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Limits: **Publication Date to 2002/10/18**

- Search History will be lost after eight hours of inactivity.
- To combine searches use # before search number, e.g., #2 AND #6.
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Search	Most Recent Queries	Time	Result
#31	Search **proteolysis and (cell culture) and CHO and** Limits: **Publication Date to 2002/10/18**	11:43:31	9
#30	Search **proteolysis and (cell culture) and CHO and Ig** Limits: **Publication Date to 2002/10/18**	11:42:43	1
#28	Search **proteolysis and (cell culture) and CHO** Limits: **Publication Date to 2002/10/18**	11:40:46	9
#27	Search **proteolysis and (cell culture)** Limits: **Publication Date to 2002/10/18**	11:40:41	109
#26	Search **proteolysis and (cell culture) and (antibody fragment)** Limits: **Publication Date to 2002/10/18**	11:40:21	4
#24	Search **(antibody fragment) and CHO and pepsin** Limits: **Publication Date to 2002/10/18**	10:44:35	2
#23	Search **(antibody fragment) and CHO** Limits: **Publication Date to 2002/10/18**	10:43:36	134
#22	Search **(antibody fragment) and (protease digestion) and CHO** Limits: **Publication Date to 2002/10/18**	10:42:55	4
#21	Search **(antibody fragment) and (enzyme digestion) and CHO** Limits: **Publication Date to 2002/10/18**	10:42:21	7
#20	Search **(antibody fragment) and (enzyme digestion)** Limits: **Publication Date to 2002/10/18**	10:42:11	435
#19	Search **antibody and CHO and (enzyme digestion)** Limits: **Publication Date to 2002/10/18**	10:40:29	37
#14	Search **antibody and CHO and (protease digestion)** Limits: **Publication Date to 2002/10/18**	10:37:39	11
#13	Search **antibody and CHO and enzyme and (protease digestion)** Limits: **Publication Date to 2002/10/18**	10:37:00	11
#10	Search **antibody and CHO and enzyme and protease and (protease digestion)** Limits: **Publication Date to 2002/10/18**	10:22:39	11
#9	Search **antibody and CHO and enzyme and protease** Limits: **Publication Date to 2002/10/18**	10:22:24	255
#8	Search **antibody and CHO and enzyme** Limits:	10:22:16	1254

Publication Date to 2002/10/18		
#7 Search **antibody and CHO** Limits: **Publication Date to 2002/10/18**	10:22:02	2998
#6 Search **antibody and CHO-DG44** Limits: **Publication Date to 2002/10/18**	10:21:49	0
#5 Search **protease and CHO-DG44** Limits: **Publication Date to 2002/10/18**	10:21:36	0
#4 Search **pepsin and CHO-DG44** Limits: **Publication Date to 2002/10/18**	10:20:49	1
#3 Search **endogenous enzyme and CHO-DG44** Limits: **Publication Date to 2002/10/18**	10:20:36	0
#2 Search **endogenous enzyme and CHO and pepsin** Limits: **Publication Date to 2002/10/18**	10:20:17	0
#1 Search **endogenous enzyme and CHO** Limits: **Publication Date to 2002/10/18**	10:20:04	578

Clear History

Jun 14 2006 10:29:54

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, June 19, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L28	(L27 and (enzyme cleavage))	38
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☐	L25	(CHO and pepsin and culture medium)	7949
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☐	L23	(L22 and pepsin digestion)	1756
☐	L22	L21 and CHO	12537
☐	L21	(L14 and (culture medium))	17756
☐	L20	(zapata gerardo)[IN]	32
☐	L19	(L18 and (endogenous enzyme))	13
☐	L18	(L17 and aspartyl protease)	245
☐	L17	(L16 and CHO)	9774
☐	L16	(L15 and pepsin)	9774
☐	L15	L14 and CHO	17660
☐	L14	(antibody fragment) or (antibody fragmentation)	38202
☐	L13	(L12 and antibody cleavage)	5
☐	L12	L11 and cho	811
☐	L11	(antibody producing cell line)	1561
☐	L10	(L7 and (pepsin digestion))	7
☐	L9	L7 and pepsin	43
☐	L8	(L7 and aspartyl protease)	1
☐	L7	L6 and antibody	319
☐	L6	(CHO adj cell adj culture)	372
☐	L5	("CHO adj cell adj culture" and antibody)	0
☐	L4	L3 and antibody	0
☐	L3	"endogenous pepsin"	4
☐	L2	"endogenous adj pepsin"	0
☐	L1	"endogenous pepsin" and "antibody fragment"	0

END OF SEARCH HISTORY

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Last logoff:  13jun06 13:25:21
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          *** ANNOUNCEMENTS ***
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NEW FILES RELEASED
***Trademarkscan - South Korea (File 655)
***Regulatory Affairs Journals (File 183)
***Index Chemicus (File 302)
***Inspec (File 202)

RESUMED UPDATING
***File 141, Reader's Guide Abstracts
                    ***
RELOADS COMPLETED
***File 516, D&B--Dun's Market Identifiers
***File 523, D&B European Dun's Market Identifiers
***File 531, American Business Directory
*** MEDLINE has been reloaded with the 2006 MeSH (Files 154 & 155)
*** The 2005 reload of the CLAIMS files (Files 340, 341, 942)
is now available online.
                                  ***
DATABASES REMOVED
***File 196, FINDEX
***File 468, Public Opinion Online (POLL)
Chemical Structure Searching now available in Prous Science Drug
Data Report (F452), Prous Science Drugs of the Future (F453),
IMS R&D Focus (F445/955), Pharmaprojects (F128/928), Beilstein
Facts (F390), Derwent Chemistry Resource (F355) and Index Chemicus
(File 302).
                    ***
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 >>>a specific database by entering HELP NEWS <file number>.<<<
 * * *

File   1:ERIC 1966-2006/May
       (c) format only 2006 Dialog

      Set  Items  Description
      ---  -----  -----------
Cost is in DialUnits
?

FILE CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ESBIOBASE
>>>"CAPLUS" is not a valid category or service name
>>>"BIOENG" is not a valid category or service name
>>>"BIOTECHNO" is not a valid category or service name
>>>"BIOTECHDS" is not a valid category or service name
>>>"ESBIOBASE" is not a valid category or service name
>>>No valid files specified
?

B 155, 159, 10, 203, 35, 5, 467, 73, 434, 34
       19jun06 09:50:16 User290558 Session D53.1
            $1.16    0.332 DialUnits File1
     $1.16  Estimated cost File1
     $0.53  INTERNET
```

```
     $1.69  Estimated cost this search
     $1.69  Estimated total session cost   0.332 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R) 1951-2006/Jun 19
         (c) format only 2006 Dialog
 *File 155: Please see HELP NEWS 154
for information about recent updates added to MEDLINE.
  File 159:Cancerlit 1975-2002/Oct
         (c) format only 2002 Dialog
 *File 159: Cancerlit is no longer updating.
Please see HELP NEWS159.
  File  10:AGRICOLA 70-2006/May
         (c) format only 2006 Dialog
  File 203:AGRIS 1974-2006/Mar
         Dist by NAL, Intl Copr. All rights reserved
  File  35:Dissertation Abs Online 1861-2006/May
         (c) 2006 ProQuest Info&Learning
  File   5:Biosis Previews(R) 1969-2006/Jun W2
         (c) 2006 The Thomson Corporation
  File 467:ExtraMED(tm) 2000/Dec
         (c) 2001 Informania Ltd.
 *File 467: F467 will close on February 1, 2006.                    7.
  File  73:EMBASE 1974-2006/Jun 19
         (c) 2006 Elsevier Science B.V.
  File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
  File  34:SciSearch(R) Cited Ref Sci 1990-2006/Jun W2
         (c) 2006 Inst for Sci Info

      Set  Items  Description
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?

S ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
         1756269  ANTIBODY
         1138819  FRAGMENT?
           10886  ANTIBODY(W)FRAGMENT?
          109272  CHO
           36498  PEPSIN
      S1       0  ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
?

S ((ANTIBODY (W) FRAGMENT) AND CHO)
         1756269  ANTIBODY
          523468  FRAGMENT
            3896  ANTIBODY(W)FRAGMENT
          109272  CHO
      S2      53  ((ANTIBODY (W) FRAGMENT) AND CHO)
?

S (PEPSIN OR (ASPARTYL (W) PROTEASE))
           36498  PEPSIN
           17027  ASPARTYL
          301755  PROTEASE
            1993  ASPARTYL(W)PROTEASE
      S3   38400  (PEPSIN OR (ASPARTYL (W) PROTEASE))
?
```

```
Set     Items   Description
S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
?

S S2 AND S3
              53  S2
           38400  S3
      S4       0  S2 AND S3
?


Set     Items   Description
S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
?

S (PROTEASE (W) (DIGEST OR DIGESTION))
          301755  PROTEASE
           24630  DIGEST
          262827  DIGESTION
      S5    4495  (PROTEASE (W) (DIGEST OR DIGESTION))
?


Set     Items   Description
S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
S5       4495   (PROTEASE (W) (DIGEST OR DIGESTION))
?

S S2 AND S5
              53  S2
            4495  S5
      S6       0  S2 AND S5
?


Set     Items   Description
S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
S5       4495   (PROTEASE (W) (DIGEST OR DIGESTION))
S6          0   S2 AND S5
?

S (ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
         1756269  ANTIBODY
          523468  FRAGMENT
            3896  ANTIBODY(W)FRAGMENT
          301755  PROTEASE
          262827  DIGESTION
            4359  PROTEASE(W)DIGESTION
      S7       5  (ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
```

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?

S (ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
         1756269  ANTIBODY
          523468  FRAGMENT
            3896  ANTIBODY(W)FRAGMENT
         2844674  ENZYME
          262827  DIGESTION
            9648  ENZYME(W)DIGESTION
      S8       0  (ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
?


Set     Items   Description
S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
S5       4495   (PROTEASE (W) (DIGEST OR DIGESTION))
S6          0   S2 AND S5
S7          5   (ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
S8          0   (ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
?

RD S7
      S9       2  RD S7  (unique items)
?

TYPE S9/FULL/1-2

  9/9/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

14777640   PMID: 14564491
 Expression of foreign proteins in Escherichia coli by fusing with an
 archaeal FK506 binding protein.
  Ideno A; Furutani M; Iwabuchi T; Iida T; Iba Y; Kurosawa Y; Sakuraba H;
Ohshima T; Kawarabayashi Y; Maruyama T
  Marine Biotechnology Institute, Kamaishi, 026-0001 Iwate, Japan.
ideno001@smile2.sekisui.co.jp
  Applied microbiology and biotechnology (Germany) Mar 2004, 64 (1)
 p99-105, ISSN 0175-7598--Print Journal Code: 8406612
  Publishing Model Print-Electronic
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: MEDLINE; Completed
  Subfile: INDEX MEDICUS
  Improper protein-folding often results in inclusion-body formation in a
protein expression system using Escherichia coli. To express such proteins
in the soluble fraction of E. coli cytoplasm, we developed an expression
system by fusing the target protein with an archaeal FK506 binding protein
(FKBP). It has been reported that an archaeal FKBP from a hyperthermophilic
archaeon, Thermococcus sp. KS-1 (TcFKBP18), possesses not only
peptidyl-prolyl cis-trans isomerase activity, but also chaperone-like
activity to enhance the refolding yield of an unfolded protein by
suppressing irreversible protein aggregation. To study the effect of this
fusion strategy with FKBP on the expression of foreign protein in E. coli,
a putative rhodanese (thiosulfate sulfurtransferase) from a
```

hyperthermophilic archaeon and two mouse antibody fragments were used as model target proteins. When they were expressed alone in E. coli, they formed insoluble aggregates. Their genes were designed to be expressed as a fusion protein by connecting them to the C-terminal end of TcFKBP18 with an oligopeptide containing a thrombin cleavage site. By fusing TcFKBP18, the expression of the target protein in the soluble fraction was significantly increased. The percentage of the soluble form in the expressed protein reached 10-28% of the host soluble proteins. After purification and protease digestion of the expressed antibody fragment-TcFKBP18 fusion protein, the cleaved antibody fragment (single-chain Fv) showed specific binding to the antigen in ELISA. This indicated that the expressed antibody fragment properly folded to the active form.

Descriptors: *Escherichia coli--genetics--GE; *Escherichia coli --metabolism--ME; *Gene Expression; *Recombinant Fusion Proteins --metabolism--ME; *Tacrolimus Binding Proteins--genetics--GE; Archaeal Proteins--genetics--GE; Bacterial Proteins--biosynthesis--BI; Cloning, Molecular; Electrophoresis, Polyacrylamide Gel; Enzyme-Linked Immunosorbent Assay; Immunoglobulin Fragments--genetics--GE; Immunoglobulin Fragments --metabolism--ME; Inclusion Bodies--metabolism--ME; Molecular Chaperones --genetics--GE; Molecular Chaperones--metabolism--ME; Muramidase--analysis --AN; Muramidase--immunology--IM; Peptidylprolyl Isomerase--genetics--GE; Peptidylprolyl Isomerase--metabolism--ME; Recombinant Fusion Proteins --analysis--AN; Solubility; Thiosulfate Sulfurtransferase--genetics--GE; Thiosulfate Sulfurtransferase--metabolism--ME

CAS Registry No.: 0 (Archaeal Proteins); 0 (Bacterial Proteins); 0 (Immunoglobulin Fragments); 0 (Molecular Chaperones); 0 (Recombinant Fusion Proteins)

Enzyme No.: EC 2.8.1.1 (Thiosulfate Sulfurtransferase); EC 3.2.1.- (hen egg lysozyme); EC 3.2.1.17 (Muramidase); EC 5.2.1.- (Tacrolimus Binding Proteins); EC 5.2.1.8 (Peptidylprolyl Isomerase)

Record Date Created: 20040309

Record Date Completed: 20040601

Date of Electronic Publication: 20031015

9/9/2 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

0015257199 BIOSIS NO.: 200500163371

An engineered chaperonin caging a guest protein: Structural insights and potential as a protein expression tool

AUTHOR: Furutani Masahiro (Reprint); Hata Jun-ichi; Shomura Yasuhito; Itami Keisuke; Yoshida Takao; Izumoto Yoshitaka; Togi Akiko; Ideno Akira; Yasunaga Takuo; Miki Kunio; Maruyama Tadashi

AUTHOR ADDRESS: Inst Res and Dev, Sekisui Chem Co Ltd, Hyakuyama 2-1, Mishima, Osaka, 6188589, Japan**Japan

AUTHOR E-MAIL ADDRESS: furutani002@sekisui.jp

JOURNAL: Protein Science 14 (2): p341-350 February 2005 2005

MEDIUM: print

ISSN: 0961-8368

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The structure of a chaperonin caging a substrate protein is not quite clear. We made engineered group H chaperonins fused with a guest protein and analyzed their structural and functional features. Themococcus sp. KS-1 chaperonin alpha-subunit (TCP) which forms an eightfold symmetric double-ring structure was used. Expression plasmids

were constructed which carried two or four TCP genes ligated head to tail in phase and a target protein gene at the 3' end of the linked TCP genes. Electron microscopy showed that the expressed gene products with the molecular sizes of Crapprx120 kDa (di-TCP) and apprx230 kDa (tetra-TCP) formed double-ring complexes similar to those of wild-type TCP. The tetra-TCP retained ATPase activity and its thermostability was significantly higher than that of the wild type. A 260-kDa fusion protein of tetra-TCP and green fluorescent protein (GFP, 27 kDa) was able to form the double-ring complexes with green fluorescence. Image analyses indicated that the GFP moiety of tetra-TCP/GFP fusion protein was accommodated in the central cavity, and tetra-TCP/GFP formed the closed-form similar to that crystallographically resolved in group II chaperonins. Furthermore, it was suggested that caging GFP expanded the cavity around the bottom. Using this tetra-TCP fusion strategy, two virus structural proteins (21-25 kDa) toxic to host cells or two antibody fragments (25-36 kDa) prone to aggregate were well expressed in the soluble, fraction of Escherichia coli. These fusion products also assembled to double-ring complexes, SU22esting encapsulation of the guest proteins. The antibody fragments liberated by site-specific protease digestion exhibited ligand-binding activities.

REGISTRY NUMBERS: 9000-83-3: ATPase; 9001-92-7: protease; 9001-90-5: protease
ENZYME COMMISSION NUMBER: EC 3.6.4.9: chaperonin; EC 3.4.21.7: protease
DESCRIPTORS:
MAJOR CONCEPTS: Cell Biology; Enzymology--Biochemistry and Molecular Biophysics; Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Thermococcaceae--Thermococcales, Extremely Thermophilic Sulfur-Metabolizers, Archaeobacteria, Bacteria, Microorganisms
ORGANISMS: Thermococcus (Thermococcaceae)
COMMON TAXONOMIC TERMS: Archaeobacteria; Bacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: ATPase; antibody fragment; chaperonin; expression plasmid; gene product; green fluorescence protein; green fluorescent protein {GFP}; group II chaperonin; protease--digestion; structural protein
MOLECULAR SEQUENCE DATABANK NUMBER: AY247032--DDBJ, EMBL, GenBank, amino acid sequence, nucleotide sequence; U10234--DDBJ, EMBL, GenBank, amino acid sequence, nucleotide sequence
GENE NAME: Thermococcus TCP gene (Thermococcaceae)
METHODS & EQUIPMENT: crystallography--crystallographic techniques, laboratory techniques; electron microscopy--imaging and microscopy techniques, laboratory techniques; image analysis--imaging and microscopy techniques, laboratory techniques
MISCELLANEOUS TERMS: thermostability
CONCEPT CODES:
02502 Cytology - General
03502 Genetics - General
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10064 Biochemistry studies - Proteins, peptides and amino acids
10802 Enzymes - General and comparative studies: coenzymes
30500 Morphology and cytology of bacteria
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
09911 Thermococcaceae
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Set Items Description

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S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
S5       4495   (PROTEASE (W) (DIGEST OR DIGESTION))
S6          0   S2 AND S5
S7          5   (ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
S8          0   (ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
S9          2   RD S7  (unique items)
?

RD S2
     S10      28  RD S2  (unique items)
?

S S10 AND PROTEASE
              28  S10
          301755  PROTEASE
     S11       1  S10 AND PROTEASE
?


Set     Items   Description
S1          0   ((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2         53   ((ANTIBODY (W) FRAGMENT) AND CHO)
S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
S5       4495   (PROTEASE (W) (DIGEST OR DIGESTION))
S6          0   S2 AND S5
S7          5   (ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
S8          0   (ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
S9          2   RD S7  (unique items)
S10        28   RD S2  (unique items)
S11         1   S10 AND PROTEASE
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TYPE S11/FULL/1

  11/9/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11317000   PMID: 9129313
 Role of high-performance liquid chromatographic protein analysis in
 developing fermentation processes for recombinant human growth hormone,
 relaxin, antibody fragments and lymphotoxin.
  Jacobson F S; Hanson J T; Wong P Y; Mulkerrin M; Deveney J; Reilly D;
Wong S C
  Department of Fermentation and Cell Culture Process Development,
Genentech, Inc., South San Francisco, CA 94080, USA.
  Journal of chromatography. A (NETHERLANDS) Feb 28 1997, 763 (1-2)
 p31-48, ISSN 0021-9673--Print Journal Code: 9318488
  Publishing Model Print
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: MEDLINE; Completed
  Subfile:  INDEX MEDICUS
  Development of efficient and reliable fermentation processes for protein
pharmaceuticals is aided by the availability of accurate quantitative and
```

qualitative product analyses. We have developed a variety of single and dual column chromatographic separations that meet the needs of process development and examples will be provided of how the resulting data has been used to optimize the culture process. For single column methods, reversed-phase chromatography has been the most versatile, permitting the reliable quantitation of many yeast, Chinese hamster ovary (CHO) cell and Escherichia coli-expressed products in the matrix of culture broth or cell extract. Analysis of secreted human growth hormone synthesized in E. coli, along with clipped and unprocessed forms, will be discussed. Another reversed-phase assay for direct analysis of a peptide product (B-chain relaxin) and its degradation products secreted into E. coli fermentation medium has allowed the purification of the responsible protease. Cation-exchange has proven extremely useful for the direct analysis of antibody fragment synthesized in E. coli, allowing the separation and quantitation of the desired Fab' and Fab'2, as well as the unwanted products of glutathione addition and translational read-through. Assay development is often complicated by the presence of host proteins with chromatographic behavior that is similar to that of the product. Commercial instrumentation now permits the facile development of multidimensional chromatographic assays. We show examples of coupled receptor affinity-reversed-phase assays for a mistranslation product and for covalent multimers of E. coli-synthesized lymphotoxin.

Descriptors: *Chromatography, High Pressure Liquid--methods--MT; *Fermentation; *Human Growth Hormone--biosynthesis--BI; *Immunoglobulin Fragments--biosynthesis--BI; *Lymphotoxin--biosynthesis--BI; *Relaxin --biosynthesis--BI; Amino Acid Sequence; Animals; CHO Cells; Cricetinae; Escherichia coli--metabolism--ME; Human Growth Hormone--analysis--AN; Humans; Immunoglobulin Fragments--analysis--AN; Lymphotoxin--analysis--AN; Recombinant Proteins--analysis--AN; Recombinant Proteins--biosynthesis--BI ; Relaxin--analysis--AN; Sequence Analysis; Technology, Pharmaceutical

CAS Registry No.: 0 (Immunoglobulin Fragments); 0 (Lymphotoxin); 0 (Recombinant Proteins); 12629-01-5 (Human Growth Hormone); 9002-69-1 (Relaxin)

Record Date Created: 19970605

Record Date Completed: 19970605

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Set	Items	Description
S1	0	((ANTIBODY (W) FRAGMENT?) AND CHO AND PEPSIN)
S2	53	((ANTIBODY (W) FRAGMENT) AND CHO)
S3	38400	(PEPSIN OR (ASPARTYL (W) PROTEASE))
S4	0	S2 AND S3
S5	4495	(PROTEASE (W) (DIGEST OR DIGESTION))
S6	0	S2 AND S5
S7	5	(ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
S8	0	(ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
S9	2	RD S7 (unique items)
S10	28	RD S2 (unique items)
S11	1	S10 AND PROTEASE

?

TYPE S10/FULL/1-28

10/9/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

19388664 PMID: 15937942

Automated in situ measurement of cell-specific antibody secretion and

laser-mediated purification for rapid cloning of highly-secreting producers.

Hanania Elie G; Fieck Annabeth; Stevens Janine; Bodzin Leon J; Palsson Bernhard O; Koller Manfred R

Cyntellect, Inc., San Diego, California, USA.

Biotechnology and bioengineering (United States) Sep 30 2005, 91 (7) p872-6, ISSN 0006-3592--Print Journal Code: 7502021

Contract/Grant No.: R44RR15374; RR; NCRR

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Cloning of highly-secreting recombinant cells is critical for biopharmaceutical manufacturing, but faces numerous challenges including the fact that secreted protein does not remain associated with the producing cell. A fundamentally new approach was developed combining in situ capture and measurement of individual cell protein secretion followed by laser-mediated elimination of all non- and poorly-secreting cells, leaving only the highest-secreting cell in a well. Recombinant cells producing humanized antibody were cultured serum-free on a capture matrix, followed by staining with fluorescently-labeled anti-human antibody fragment. A novel, automated, high-throughput instrument (called LEAP) was used to image and locate every cell, quantify the cell-associated and secreted antibody (surrounding each cell), eliminate all undesired cells from a well via targeted laser irradiation, and then track clone outgrowth and stability. Temporarily sparing an island of helper cells around the clone of interest improved cloning efficiency (particularly when using serum-free medium), and helper cells were easily eliminated with the laser after several days. The in situ nature of this process allowed several serial sub-cloning steps to be performed within days of one another, resulting in rapid generation of clonal populations with significantly increased and more stable, homogeneous antibody secretion. Cell lines with specific antibody secretion rates of > 50 pg/cell per day (in static batch culture) were routinely obtained as a result of this cloning approach, often times representing up to 20% of the clones screened. Copyright 2005 Wiley Periodicals, Inc

Descriptors: *Antibodies--genetics--GE; *Antibody-Producing Cells; *Cell Separation--methods--MT; *Cloning, Molecular--methods--MT; Animals; Antibodies--analysis--AN; CHO Cells; Cell Adhesion; Cell Line; Cricetinae; Humans; Hybridomas; Laser Scanning Cytometry; Lasers; Mice; Microscopy, Fluorescence; Recombinant Proteins--analysis--AN; Recombinant Proteins --genetics--GE; Research Support, N.I.H., Extramural; Research Support, U.S. Gov't, P.H.S.; Staining and Labeling

CAS Registry No.: 0 (Antibodies); 0 (Recombinant Proteins)

Record Date Created: 20050830

Record Date Completed: 20051205

10/9/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

15419287 PMID: 15833872

Target cell-restricted apoptosis induction of acute leukemic T cells by a recombinant tumor necrosis factor-related apoptosis-inducing ligand fusion protein with specificity for human CD7.

Bremer Edwin; Samplonius Douwe F; Peipp Matthias; van Genne Linda; Kroesen Bart-Jan; Fey Georg H; Gramatzki Martin; de Leij Lou F M H;

Helfrich Wijnand
Laboratory for Tumor Immunology, Department of Pathology and Laboratory Medicine, Section Medical Biology, University Hospital Groningen, Groningen University Institute for Drug Exploration, 9713 GZ Groningen, the Netherlands.
Cancer research (United States) Apr 15 2005, 65 (8) p3380-8, ISSN 0008-5472--Print Journal Code: 2984705R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Current treatment of human T-cell leukemia and lymphoma is predominantly limited to conventional cytotoxic therapy and is associated with limited therapeutic response and significant morbidity. Therefore, more potent and leukemia-specific therapies with favorable toxicity profiles are urgently needed. Here, we report on the construction of a novel therapeutic fusion protein, scFvCD7:sTRAIL, designed to induce target antigen-restricted apoptosis in human T-cell tumors. ScFvCD7:sTRAIL consists of the death-inducing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) genetically linked to an scFv antibody fragment specific for the T-cell surface antigen CD7. Treatment with scFvCD7:sTRAIL induced potent CD7-restricted apoptosis in a series of malignant T-cell lines, whereas normal resting leukocytes, activated T cells, and vascular endothelial cells (human umbilical vein endothelial cells) showed no detectable apoptosis. The apoptosis-inducing activity of scFvCD7:sTRAIL was stronger than that of the immunotoxin scFvCD7:ETA. In mixed culture experiments with CD7-positive and CD7-negative tumor cells, scFvCD7:sTRAIL induced very potent bystander apoptosis of CD7-negative tumor cells. In vitro treatment of blood cells freshly derived from T-acute lymphoblastic leukemia patients resulted in marked apoptosis of the malignant T cells that was strongly augmented by vincristin. In conclusion, scFvCD7:sTRAIL is a novel recombinant protein causing restricted apoptosis in human leukemic T cells with low toxicity for normal human blood and endothelial cells.
Descriptors: *Antigens, CD7--immunology--IM; *Apoptosis--drug effects--DE ; *Immunotoxins--pharmacology--PD; *Leukemia, T-Cell, Acute--drug therapy --DT; *Membrane Glycoproteins--pharmacology--PD; *Recombinant Fusion Proteins--pharmacology--PD; *Tumor Necrosis Factor-alpha--pharmacology--PD ; Animals; Antibodies, Monoclonal--genetics--GE; Antibodies, Monoclonal --immunology--IM; Antibodies, Monoclonal--pharmacology--PD; Antigens, CD7 --genetics--GE; Apoptosis--immunology--IM; Apoptosis Regulatory Proteins; CHO Cells; Cell Line, Tumor; Cricetinae; Drug Synergism; Epitopes; Humans; Immunoglobulin Fragments--genetics--GE; Immunoglobulin Fragments --immunology--IM; Immunoglobulin Fragments--pharmacology--PD; Immunotoxins --genetics--GE; Immunotoxins--immunology--IM; Jurkat Cells--cytology--CY; Jurkat Cells--drug effects--DE; Leukemia, T-Cell, Acute--immunology--IM; Leukemia, T-Cell, Acute--pathology--PA; Membrane Glycoproteins--genetics --GE; Recombinant Fusion Proteins--genetics--GE; Recombinant Fusion Proteins--immunology--IM; Research Support, Non-U.S. Gov't; T-Lymphocytes --drug effects--DE; T-Lymphocytes--immunology--IM; T-Lymphocytes --pathology--PA; Tumor Necrosis Factor-alpha--genetics--GE; Vincristine --pharmacology--PD
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens, CD7); 0 (Apoptosis Regulatory Proteins); 0 (Epitopes); 0 (Immunoglobulin Fragments); 0 (Immunotoxins); 0 (Membrane Glycoproteins); 0 (Recombinant Fusion Proteins); 0 (TNF-related apoptosis-inducing ligand) ; 0 (Tumor Necrosis Factor-alpha); 57-22-7 (Vincristine)
Record Date Created: 20050418
Record Date Completed: 20050603

10/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

15333751 PMID: 15713482
A novel bispecific tetravalent antibody fusion protein to target costimulatory activity for T-cell activation to tumor cells overexpressing ErbB2/HER2.
Biburger Markus; Weth Robert; Wels Winfried S
Chemotherapeutisches Forschungsinstitut Georg-Speyer-Haus, Paul-Ehrlich-Strasse 42-44, 60596 Frankfurt am Main, Germany.
Journal of molecular biology (England) Mar 11 2005, 346 (5) p1299-311, ISSN 0022-2836--Print Journal Code: 2985088R
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Persistent activation of T-lymphocytes requires two signals: one is initiated by T-cell receptor binding to antigenic peptide presented by MHC molecules. In addition, binding of the B7 family members CD80 or CD86 on professional antigen presenting cells to CD28 on T cells is considered to provide an important costimulatory signal. Activation without costimulation induces T-cell unresponsiveness or anergy. To selectively localize costimulatory activity to the surface of tumor cells and enhance activation of tumor-specific T cells, we have developed a novel molecular design for bispecific costimulatory proteins with antibody-like structure. Within a single polypeptide chain we have assembled the IgV-like, CD28-binding domain of human CD86 (CD86(111)) together with hinge, CH2 and CH3 domains of human IgG1, and the scFv(FRP5) antibody fragment which recognizes the ErbB2 (HER2) protooncogene present at high levels on the surface of many human tumor cells. Upon expression in the yeast Pichia pastoris, the resulting CD86(111)-IgG-scFv(FRP5) protein could be purified as a homodimeric, tetravalent molecule from culture supernatants using single-step affinity chromatography. Bispecific binding of the molecule to ErbB2 on the surface of tumor cells and to the B7 counter receptor CTLA-4 was demonstrated by FACS analysis. Potent costimulatory activity of chimeric CD86(111)-IgG-scFv(FRP5) was confirmed by its ability to stimulate the proliferation of primary human lymphocytes pre-activated by low concentrations of anti-CD3 antibody. Our results suggest that such multivalent soluble proteins which combine specific targeting to tumor cells with costimulatory activity may become useful tools to elicit and/or improve T-cell mediated, tumor-specific immune responses.
Descriptors: *Antibodies, Bispecific--immunology--IM; *HLA-B7 Antigen --immunology--IM; *Neoplasms--metabolism--ME; *Peptide Fragments --immunology--IM; *Receptor, erbB-2--immunology--IM; *Recombinant Fusion Proteins--immunology--IM; *T-Lymphocytes--immunology--IM; Animals; Antigens, CD--immunology--IM; Antigens, CD--metabolism--ME; Antigens, CD28 --immunology--IM; Antigens, CD28--metabolism--ME; Antigens, CD3 --immunology--IM; Antigens, CD86; Antigens, Differentiation; CHO Cells; Cell Proliferation; Cricetinae; Humans; Immunoglobulin Fragments--genetics --GE; Immunoglobulin Fragments--immunology--IM; Immunoglobulin Fragments --metabolism--ME; Immunoglobulin Variable Region--genetics--GE; Immunoglobulin Variable Region--immunology--IM; Immunoglobulin Variable Region--metabolism--ME; Lymphocyte Activation; Membrane Glycoproteins --immunology--IM; Membrane Glycoproteins--metabolism--ME; Neoplasms --genetics--GE; Neoplasms--immunology--IM; Peptide Fragments--metabolism

--ME; Pichia--immunology--IM; Protein Binding; Protein Structure, Tertiary; Recombinant Fusion Proteins--genetics--GE; Recombinant Fusion Proteins --metabolism--ME; Research Support, Non-U.S. Gov't
CAS Registry No.: 0 (Antibodies, Bispecific); 0 (Antigens, CD); 0 (Antigens, CD28); 0 (Antigens, CD3); 0 (Antigens, CD86); 0 (Antigens, Differentiation); 0 (CD86 protein, human); 0 (HLA-B7 Antigen); 0 (Immunoglobulin Fragments); 0 (Immunoglobulin Variable Region); 0 (Membrane Glycoproteins); 0 (Peptide Fragments); 0 (Recombinant Fusion Proteins); 0 (cytotoxic T-lymphocyte antigen 4); 0 (immunoglobulin Fv)
Enzyme No.: EC 2.7.1.112 (Receptor, erbB-2)
Record Date Created: 20050216
Record Date Completed: 20050329
Date of Electronic Publication: 20050122

10/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

15224088 PMID: 15485827
Modulation of the M2 muscarinic acetylcholine receptor activity with monoclonal anti-M2 receptor antibody fragments.
Peter Jean-Christophe; Wallukat Gerd; Tugler Jean; Maurice Damien; Roegel Jean-Christophe; Briand Jean-Paul; Hoebeke Johan
CNRS, Unite Propre de Recherche 9021, Institut de Biologie Moleculaire et Cellulaire, Laboratory of Therapeutical Chemistry and Immunology, 15 rue Rene Descartes, F-67084 Strasbourg, France.
Journal of biological chemistry (United States) Dec 31 2004, 279 (53) p55697-706, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Antibodies directed against the second extracellular loop of G protein-coupled receptors are known to have functional activities. From a partial agonist monoclonal antibody directed against the M2 muscarinic receptor, we constructed and produced a single chain variable fragment with high affinity for its target epitope. The fragment is able to recognize its receptor on Chinese hamster ovary cells transfected with the M2 muscarinic acetylcholine receptor to block the effect of carbachol on this receptor and to exert an inverse agonist activity on the basal activity of the receptor. The antibody fragment is also able to increase the basal rhythm of cultured neonatal rat cardiomyocytes and to inhibit in a non-competitive manner the negative chronotropic effect of carbachol. This antibody fragment is able to exert its inverse agonist activity in vivo on mouse heart activity. The immunological strategy presented here could be useful to develop specific allosteric inverse agonist reagents for G protein-coupled receptors.
Descriptors: *Receptor, Muscarinic M2--chemistry--CH; Allosteric Site; Amino Acid Sequence; Animals; Antibodies, Monoclonal--chemistry--CH; Base Sequence; Binding, Competitive; Blotting, Western; CHO Cells; Carbachol --pharmacology--PD; Cells, Cultured; Cricetinae; Dose-Response Relationship, Drug; Dose-Response Relationship, Immunologic; Electrophoresis, Polyacrylamide Gel; Epitopes--chemistry--CH; Escherichia coli--metabolism--ME; Immunoglobulin Fab Fragments; Immunohistochemistry; Mice; Models, Molecular; Molecular Sequence Data; Myocytes, Cardiac --metabolism--ME; Nucleotides--chemistry--CH; Peptides--chemistry--CH; Protein Structure, Tertiary; Rats; Receptor, Muscarinic M2--metabolism--ME;

Receptors, Cholinergic--chemistry--CH; Research Support, Non-U.S. Gov't; Sequence Homology, Amino Acid; Surface Plasmon Resonance; Time Factors; Transfection
Molecular Sequence Databank No.: GENBANK/AJ746180
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Epitopes); 0 (Immunoglobulin Fab Fragments); 0 (Nucleotides); 0 (Peptides); 0 (Receptor, Muscarinic M2); 0 (Receptors, Cholinergic); 51-83-2 (Carbachol)
Record Date Created: 20041223
Record Date Completed: 20050225
Date of Electronic Publication: 20041014

10/9/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

15182783 PMID: 15555554
2D7 diabody bound to the alpha2 domain of HLA class I efficiently induces caspase-independent cell death against malignant and activated lymphoid cells.
Kimura Naoki; Kawai Shigeto; Kinoshita Yasuko; Ishiguro Takahiro; Azuma Yumiko; Ozaki Shuji; Abe Masahiro; Sugimoto Masamichi; Hirata Yuichi; Orita Tetsuro; Okabe Hisafumi; Matsumoto Toshio; Tsuchiya Masayuki
Genome Antibody Product Research Department, Chugai Pharmaceutical Co., Ltd., Japan. kimuranok@chugai-pharm.co.jp
Biochemical and biophysical research communications (United States) Dec 24 2004, 325 (4) p1201-9, ISSN 0006-291X--Print Journal Code: 0372516
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A mouse monoclonal antibody (2D7 mAb), which specifically bound to the alpha2 domain of HLA class I, rapidly induces cell aggregation accompanied by weak cytotoxicity against ARH-77 cells, suggesting that 2D7 mAb had a potential for agonist antibody. In order to enhance this cytotoxicity, 2D7 mAb was engineered to be a small bivalent antibody fragment, 2D7 diabody. The resultant 2D7 diabody showed a strong cytotoxicity against ARH-77 cells. As a notable characteristic feature, the lethal effect of 2D7 diabody was quite rapid, mediated by a caspase-independent death pathway. Furthermore, 2D7 diabody also showed cytotoxicity against several leukemia and lymphoma cell lines, and mitogen-activated peripheral blood mononuclear cells (PBMC), but not for normal resting PBMC and adherent cell lines such as HUVEC. These results suggest that 2D7 diabody could be expected as a novel therapeutic antibody for hematological malignancies as well as inflammatory diseases.
Descriptors: *Antibodies, Monoclonal--administration and dosage--AD; *Antibodies, Monoclonal--immunology--IM; *Apoptosis--immunology--IM; *Histocompatibility Antigens Class I--immunology--IM; *Leukocytes, Mononuclear--drug effects--DE; *Leukocytes, Mononuclear--immunology--IM; *Multiple Myeloma--immunology--IM; Animals; Apoptosis--drug effects--DE; CHO Cells; Caspases--immunology--IM; Cell Line, Tumor; Cells, Cultured; Comparative Study; Cricetinae; Cricetulus; Dose-Response Relationship, Drug ; Humans; Multiple Myeloma--pathology--PA; Protein Binding; Protein Structure, Tertiary
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Histocompatibility Antigens Class I)
Enzyme No.: EC 3.4.22.- (Caspases)

Record Date Created: 20041123
Record Date Completed: 20050118

10/9/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14761157 PMID: 14985099
A bivalent single-chain Fv fragment against CD47 induces apoptosis for leukemic cells.
Kikuchi Yasufumi; Uno Shinsuke; Yoshimura Yasushi; Otabe Koji; Iida Shin-ichiro; Oheda Masayoshi; Fukushima Naoshi; Tsuchiya Masayuki
Chugai Pharmaceutical Co., Ltd., Fuji-Gotemba Research Laboratories, 1-135 Komakado, Gotemba-shi, Shizuoka-ken 412-8513, Japan. kikuchiysf@chugai-pharm.co.jp
Biochemical and biophysical research communications (United States) Mar 19 2004, 315 (4) p912-8, ISSN 0006-291X--Print Journal Code: 0372516
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
We constructed a single-chain antibody fragment (scFv) of murine monoclonal antibody, MABL, which specifically bound to human CD47 (hCD47) and induced apoptosis of the leukemic cells. The scFv of MABL antibody with a 15-residue linker (MABL scFv-15) formed both dimer (Mr 50 kDa) and monomer (Mr 25 kDa). Both MABL scFv-15 dimer and monomer had binding activity for hCD47. MABL scFv-15 dimer strongly induced apoptosis of hCD47-introduced mouse leukemic cells in vitro and exhibited anti-tumor effect in a myeloma transplanted mice model. However, MABL scFv-15 monomer scarcely exhibited these activities. These results strongly demonstrate that the ligation of CD47 antigen by two antigen-binding sites of MABL dimer is needed for inducing apoptosis. The parent MABL antibody caused hemagglutination due to the CD47 expressed on erythrocytes. Interestingly, MABL scFv-15 dimer did not cause hemagglutination. This apoptosis-inducing dimer appears to be a lead candidate for novel leukemic therapy.
Tags: Male
Descriptors: *Antigens, CD--immunology--IM; *Apoptosis--drug effects--DE; *Carrier Proteins--immunology--IM; *Immunoglobulin Fragments--immunology --IM; *Immunoglobulin Fragments--pharmacology--PD; *Leukemia L1210 --pathology--PA; Animals; Antibodies, Monoclonal--chemistry--CH; Antigens, CD--metabolism--ME; Antigens, CD47; Binding Sites; CHO Cells; Carrier Proteins--metabolism--ME; Cell Line, Tumor; Cell Survival--drug effects--DE ; Cricetinae; Dimerization; Flow Cytometry--methods--MT; Hemagglutination Tests; Humans; Immunoglobulin Fragments--genetics--GE; Immunoglobulin Fragments--metabolism--ME; Immunoglobulin G--blood--BL; Immunoglobulin Variable Region--genetics--GE; Immunoglobulin Variable Region--immunology --IM; Immunoglobulin Variable Region--metabolism--ME; Immunoglobulin Variable Region--pharmacology--PD; Leukemia L1210--immunology--IM; Leukemia L1210--therapy--TH; Mice; Mice, SCID; Multiple Myeloma --immunology--IM; Multiple Myeloma--pathology--PA; Multiple Myeloma --therapy--TH; Neoplasm Transplantation--immunology--IM; Recombinant Proteins--genetics--GE; Recombinant Proteins--immunology--IM; Recombinant Proteins--metabolism--ME; Recombinant Proteins--pharmacology--PD
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens, CD); 0 (Antigens, CD47); 0 (CD47 protein, human); 0 (Carrier Proteins); 0 (Cd47 protein, mouse); 0 (Immunoglobulin Fragments); 0 (Immunoglobulin G); 0 (Immunoglobulin Variable Region); 0 (Recombinant Proteins)

Record Date Created: 20040226
Record Date Completed: 20040420

10/9/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14709764 PMID: 16233398
Production of anti-prion scFv-Fc fusion proteins by recombinant animal cells.
Ono Ken-Ichiro; Kamihira Masamichi; Kuga Yuko; Matsumoto Hiroyuki; Hotta Akitsu; Itoh Toshinari; Nishijima Ken-Ichi; Nakamura Naoto; Matsuda Haruo; Iijima Shinji
Department of Biotechnology, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan.
Journal of bioscience and bioengineering (Japan) 2003, 95 (3) p231-8, ISSN 1389-1723--Print Journal Code: 100888800
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: PubMed not MEDLINE
We constructed a replication-defective retroviral vector plasmid for the expression of a single-chain antibody fragment (scFv), derived from a chicken anti-human prion protein monoclonal antibody, fused with the Fc region of human IgG1. CHO-K1 and NS-1 cells were transformed with the viral vector pseudotyped with vesicular stomatitis virus G protein (VSV-G), and scFv-Fc producer clones were established. Among the established clones, CHO-2A9 cells produced a large amount of the product with an antibody-like dimerized structure in serum-free culture that facilitated the purification of scFv-Fc. The scFv-Fc specifically recognized the epitope sequence of prion protein in solid-phase enzyme-linked immunosorbent assay (ELISA) and Western blot analysis. The injection test into quails revealed that the scFv became more stable in vivo by fusion with the Fc region. The scFv-Fc will be a useful tool for the detection of mammalian prion proteins.
Record Date Created: 20051019
Record Date Completed: 20051108

10/9/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14618804 PMID: 14659901
A new epitope tag from hepatitis B virus preS1 for immunodetection, localization and affinity purification of recombinant proteins.
Oh Mee Sook; Kim Keun Soo; Jang Young Kug; Maeng Cheol Young; Min Soon Hong; Jang Myeong Hee; Yoon Sun Ok; Kim Jung Hee; Hong Hyo Jeong
R and D Center, Aprogen, Inc., Bio Venture Center No. 311, Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yuseong, 305-600 Taejon, South Korea.
Journal of immunological methods (Netherlands) Dec 2003, 283 (1-2) p77-89, ISSN 0022-1759--Print Journal Code: 1305440
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Previously, a murine monoclonal antibody (mAb) KR127 (IgG2a/kappa) that binds specifically to the preS1 of hepatitis B virus (HBV) was generated and the fine epitope was mapped to amino acids (aa) 37-45 (NSNNPDWDF). In this current study, the epitope in combination with KR127 was tested for protein tagging. Initially, to evaluate the importance of each residue of the KR127 epitope in antibody binding, alanine substitution mutants of the epitope were constructed and characterized for KR127 binding by immunoblot analysis and competition ELISA. The results showed that substitution of Ser(38) by alanine (S38A) increased the affinity to KR127. The mutated epitope (NANNPDWDF), designated S1 tag, was fused to the amino (N)- or carboxyl (C)-terminus of three human recombinant proteins, soluble B lymphocyte stimulator (sBLyS), the N-terminal domain of thrombopoietin (nTPO), and a mitochondrial ribosomal protein (CGI-113) for expression in mammalian cells, while it was fused to the N- or C-terminus of two proteins, a single-chain antibody fragment (ScFv) and the carboxyl-terminal domain (PAc) of the protective antigen of Bacillus anthracis for expression in Escherichia coli. The immunodetection, immunoprecipitation, and affinity purification of the expressed S1-tagged proteins by KR127 were successfully demonstrated. In addition, a KR127 mutant (AP2) with higher affinity, K(d) (0.9 nM), for the S1 tag compared to that (20 nM) of KR127 was obtained by mutational analysis of the heavy chain CDR3 (HCDR3) of KR127. The AP2 antibody was 4-fold more sensitive in detecting the S1-tagged protein than KR127. The S1 tag-KR127 or AP2 combination could be universally used for monitoring protein expression, localizing proteins, and protein purification, as well as studying protein interactions.

Descriptors: *Antibodies, Monoclonal--immunology--IM; *Epitopes --immunology--IM; *Hepatitis B Surface Antigens--immunology--IM; *Protein Precursors--immunology--IM; *Recombinant Proteins--analysis--AN; Animals; CHO Cells; Chromatography, Affinity; Cricetinae; Escherichia coli--genetics --GE; Precipitin Tests; Recombinant Proteins--isolation and purification --IP; Research Support, Non-U.S. Gov't; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Epitopes); 0 (Hepatitis B Surface Antigens); 0 (Protein Precursors); 0 (Recombinant Proteins); 0 (presurface protein 1, hepatitis B surface antigen)

Record Date Created: 20031208

Record Date Completed: 20040122

10/9/9 (Item 9 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

14413817 PMID: 12881037

Adhesion of monocytes to medical steel as used for vascular stents is mediated by the integrin receptor Mac-1 (CD11b/CD18; alphaM beta2) and can be inhibited by semiconductor coating.

Schuler Pia; Assefa Dawit; Ylanne Jari; Basler Nicole; Olschewski Manfred ; Ahrens Ingo; Nordt Thomas; Bode Christoph; Peter Karlheinz

Department of Cardiology and Angiology, University of Freiburg, Freiburg, Germany.

Cell communication & adhesion (England) Jan-Feb 2003, 10 (1) p17-26, ISSN 1541-9061--Print Journal Code: 101096596

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Implantation of stents into stenosed arteries helps to restore normal blood flow in ischemic organs. However, limited biocompatibility of the

applied medical steel can cause acute thrombosis and long-term restenosis. Adhesion of monocytes to stent metal may participate in those acute and long-term complications of stent placement. Based on described prominent electrochemical properties of the interaction between the monocyte integrin receptor Mac-1 and its various ligands, we hypothesized, that this receptor is a central mediator of monocyte adhesion to stent metal and that semiconductor coating of medical steel reduces monocyte adhesion. Adhesion of monocytes on L-316 stainless steel was directly evaluated by light microscopy. Mac-1 could be identified as mediator of monocyte adhesion, since cell adhesion could be blocked by anti-Mac-1-antibodies, including the cross-reacting anti-GPIIb/IIIa antibody fragment abciximab. To further prove the central role of Mac-1, two CHO cell lines were generated expressing recombinant Mac-1 either as wild type, resulting in a low affinity receptor, or mutant with a GFFKR deletion of the alpha(M) subunit, resulting in a high affinity receptor. Indeed, adhesion was specific for Mac-1 and dependent on the affinity state of this integrin. Finally, we could demonstrate that Mac-1-mediated adhesion of monocytes to stents can be significantly inhibited by silicon carbide coating of the stent metal. In conclusion, the integrin Mac-1 and its affinity state could be identified as major mediators of monocyte adhesion on medical steel. As therapeutic strategies, the blockade of Mac-1 by antibodies or silicon carbide coating of steel inhibits monocyte adhesion on stents.

Descriptors: *Antigens, CD11b--physiology--PH; *Monocytes--cytology--CY; *Stainless Steel--chemistry--CH; *Stents; Adhesiveness; Analysis of Variance; Animals; Antigens, CD11b--metabolism--ME; Antigens, CD18 --biosynthesis--BI; Biocompatible Materials--chemistry--CH; CHO Cells; Carbon Compounds, Inorganic--chemistry--CH; Cell Adhesion; Cell Line; Cricetinae; Electrochemistry; Flow Cytometry; Humans; Monocytes--metabolism --ME; Peptides--chemistry--CH; Semiconductors; Silicon Compounds--chemistry --CH

CAS Registry No.: 0 (Antigens, CD11b); 0 (Antigens, CD18); 0 (Biocompatible Materials); 0 (Carbon Compounds, Inorganic); 0 (Peptides); 0 (Silicon Compounds); 12597-68-1 (Stainless Steel); 409-21-2 (silicon carbide)

Record Date Created: 20030725

Record Date Completed: 20040428

10/9/10 (Item 10 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

14037064 PMID: 12459375

An antibody-calmodulin fusion protein reveals a functional dependence between macromolecular isoelectric point and tumor targeting performance.

Melkko Samu; Halin Cornelia; Borsi Laura; Zardi Luciano; Neri Dario

Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology Zurich, Zurich, Switzerland.

International journal of radiation oncology, biology, physics (United States) Dec 1 2002, 54 (5) p1485-90, ISSN 0360-3016--Print

Journal Code: 7603616

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

PURPOSE: Human monoclonal antibodies are promising agents for the development of improved anticancer therapeutics, because, unlike low-molecular-weight chemotherapeutic agents, they can selectively localize

to solid tumors. In particular, the scFv(L19) antibody fragment, specific for the EDB domain of fibronectin, a marker of angiogenesis, has demonstrated an impressive tumor targeting performance in a variety of tumor-bearing animals and in patients with cancer. The purpose of this study was to develop a tumor pretargeting strategy, based on a novel anti-EDB fusion protein. METHODS AND MATERIALS: We have fused the scFv(L19) to calmodulin, a small acidic protein for which specific binding peptides with a dissociation constant in the picomolar range are available. The resulting fusion protein has been expressed in mammalian cells and purified to homogeneity, before being characterized by quantitative biodistribution analysis in mice bearing the F9 murine teratocarcinoma. RESULTS: Surprisingly, we have found that the fusion of scFv(L19) to calmodulin completely abrogated the tumor targeting ability of the antibody in vivo, although both scFv(L19) and calmodulin moieties within the fusion protein retained unaltered binding affinities toward their respective ligand. Furthermore, a systematic analysis of 13 derivatives of scFv(L19) recently produced in our laboratories showed that the 10 derivatives that retain the tumor targeting ability of the parental antibody have isoelectric points (pI) between 5.0 and 9.0, whereas scFv(L19)-calmodulin (pI = 4.49) and two other derivatives of scFv(L19) with pI >9.0 were unable to target tumors in vivo. CONCLUSIONS: Because the EDB domain of fibronectin is a component of the modified extracellular matrix, predominantly located at the abluminal side of tumor blood vessels, our data suggest that extreme pI values of antibody-based pharmaceuticals may inhibit protein extravasation, perhaps by virtue of electrostatic interactions with endothelial cells and/or components of the extracellular matrix.

Descriptors: *Calmodulin--chemistry--CH; Animals; Antineoplastic Agents --metabolism--ME; CHO Cells; Calmodulin--metabolism--ME; Cloning, Molecular ; Cricetinae; Enzyme-Linked Immunosorbent Assay; Humans; Hydrogen-Ion Concentration; Iodine Radioisotopes--metabolism--ME; Isoelectric Focusing; Mice; Models, Biological; Neovascularization, Pathologic; Protein Structure, Tertiary; Recombinant Fusion Proteins--chemistry--CH; Recombinant Fusion Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; Tissue Distribution; Tumor Cells, Cultured

CAS Registry No.: 0 (Antineoplastic Agents); 0 (Calmodulin); 0 (Iodine Radioisotopes); 0 (Recombinant Fusion Proteins)

Record Date Created: 20021202

Record Date Completed: 20030102

10/9/11 (Item 11 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

13206557 PMID: 11340579

Fusion proteins of B7.1 and a carcinoembryonic antigen (CEA)-specific antibody fragment opsonize CEA-expressing tumor cells and coactivate T-cell immunity.

Hoffmann P; Mueller N; Shively J E; Fleischer B; Neumaier M

Medical Clinic, Department of Clinical Chemistry, University Hospital Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany.

International journal of cancer. Journal international du cancer (United States) Jun 1 2001, 92 (5) p725-32, ISSN 0020-7136--Print

Journal Code: 0042124

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Genetic engineering can be used to generate antigen-specific molecules for improved tumor immunotherapy. We have constructed genes coding for fusion proteins consisting of a high-affinity antibody single-chain antibody fragment (scFv) specific for the human carcinoembryonic antigen (CEA) and the costimulation domain of the murine B7.1 molecule (mB7.1) linked to the antibody moiety by an IgG3 peptide linker. The hybrid genes were constructed in 2 orientations, one with the scFv located N-terminal to mB7.1 and one vice versa. Soluble proteins were expressed by CHO cells, purified using anti-idiotype-affinity chromatography and characterized by tumor-cell binding and costimulation activity. When tumor cells expressing CEA on the cell membrane were opsonized with the CEA-specific costimulators, both fusion proteins specifically stimulated murine T-cell preparations to proliferate in a similar manner. Our data suggest that "costimulation coating" of tumor cells may be a suitable approach for activation of a sustained cellular antitumor response. It also provides the opportunity to increase tumor immunogenicity using easily generated soluble fusion proteins that advantageously link biological functions of both the humoral and the cellular arm of the specific immune system. Copyright 2001 Wiley-Liss, Inc.

Descriptors: *Antigens, CD80--therapeutic use--TU; *Carcinoembryonic Antigen--immunology--IM; *Immunoglobulin Fragments--therapeutic use--TU; *Lymphocyte Activation; *Neoplasms, Experimental--drug therapy--DT; *Recombinant Fusion Proteins--therapeutic use--TU; *T-Lymphocytes --immunology--IM; Animals; Base Sequence; Flow Cytometry; Lymphocyte Culture Test, Mixed; Mice; Molecular Sequence Data; Neoplasms, Experimental --immunology--IM; Phagocytosis; Research Support, Non-U.S. Gov't; Tumor Cells, Cultured

CAS Registry No.: 0 (Antigens, CD80); 0 (Carcinoembryonic Antigen); 0 (Immunoglobulin Fragments); 0 (Recombinant Fusion Proteins); 0 (immunoglobulin Fv)

Record Date Created: 20010507

Record Date Completed: 20010524

10/9/12 (Item 12 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

13077547 PMID: 11211146

Engineering and characterization of a novel fusion protein incorporating B7.2 and an anti-ErbB-2 single-chain antibody fragment for the activation of Jurkat T cells.

Marshall K W; Marks J D

Department of Anesthesia, University of California, San Francisco, USA.

Journal of immunotherapy (Hagerstown, Md. - 1997) (United States) Jan-Feb 2001, 24 (1) p27-36, ISSN 1524-9557--Print Journal Code: 9706083

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The provision of the T-cell costimulatory molecule B7 to tumor cells can be an effective way to trigger a tumor-specific cytolytic T-cell response. One way to provide B7 to tumor cells would be to couple an antitumor antibody directly to B7. Such a molecule should target tumors displaying antigen and provide the costimulatory signal to T cells, resulting in the initiation of an antitumor T-cell response. To this end, a fusion protein was designed that incorporates a single-chain antibody fragment (scFv) to

erbB-2 (Her2/neu), an oncogene product overexpressed by 30% to 50% of breast carcinomas, and the ECD of B7-2 (CD86). This fusion protein, expressed and purified from Pichia pastoris, was shown to retain binding activity to both counter receptors, erbB-2 and CD28. The fusion protein was also shown to target erbB-2-positive tumor cells and to deliver a CD28-specific T-cell costimulatory signal. These results suggest that a fusion protein engineered to target tumor cells and signal T cells for activation may be an effective means of cancer immunotherapy. Further studies should be performed to characterize the fusion protein in erbB-2 tumor-bearing mice for in vivo tumor targeting, biodistribution, and efficacy.

Descriptors: *Antigens, CD--chemistry--CH; *Antigens, CD--genetics--GE; *Immunoglobulin Variable Region--chemistry--CH; *Immunoglobulin Variable Region--genetics--GE; *Lymphocyte Activation; *Membrane Glycoproteins --chemistry--CH; *Membrane Glycoproteins--genetics--GE; *Receptor, erbB-2 --genetics--GE; *Receptor, erbB-2--immunology--IM; *Recombinant Fusion Proteins--chemical synthesis--CS; Amino Acid Sequence; Animals; Antigens, CD--metabolism--ME; Antigens, CD86; CHO Cells; Cricetinae; Humans; Immunoglobulin Variable Region--metabolism--ME; Jurkat Cells; Kinetics; Lymphocyte Activation--genetics--GE; Membrane Glycoproteins--metabolism--ME ; Molecular Sequence Data; Protein Binding--genetics--GE; Protein Binding --immunology--IM; Protein Engineering--methods--MT; Rabbits; Receptor, erbB-2--metabolism--ME; Recombinant Fusion Proteins--biosynthesis--BI; Recombinant Fusion Proteins--isolation and purification--IP; Recombinant Fusion Proteins--metabolism--ME; Signal Transduction--genetics--GE; Signal Transduction--immunology--IM; Surface Plasmon Resonance; Transfection

CAS Registry No.: 0 (Antigens, CD); 0 (Antigens, CD86); 0 (CD86 protein, human); 0 (Immunoglobulin Variable Region); 0 (Membrane Glycoproteins); 0 (Recombinant Fusion Proteins)

Enzyme No.: EC 2.7.1.112 (Receptor, erbB-2)

Record Date Created: 20010208

Record Date Completed: 20010322

10/9/13 (Item 13 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

12405276 PMID: 10338505

Disruption of anthrax toxin binding with the use of human antibodies and competitive inhibitors.

Cirino N M; Sblattero D; Allen D; Peterson S R; Marks J D; Jackson P J; Bradbury A; Lehnert B E

Life Sciences Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA.

Infection and immunity (UNITED STATES) Jun 1999, 67 (6) p2957-63, ISSN 0019-9567--Print Journal Code: 0246127

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

The protective antigen (PA83) of Bacillus anthracis is integral to the mechanism of anthrax toxicity. We have isolated a human single-chain Fv antibody fragment (scFv) that blocks binding of a fluorescently tagged protective antigen (PA) moiety to cell surface receptors. Several phage-displayed scFv were isolated from a naive library biopanned against PA83. Soluble, monomeric scFv were characterized for affinity and screened for their capacity to disrupt receptor-mediated binding of PA. Four unique

scFv bound to PA83, as determined by surface plasmon resonance, the tightest binder exhibiting a Kd of 50 nM. Two scFv had similar affinities for natural PA83 and a novel, recombinant, 32-kDa carboxy-terminal PA fragment (PA32). Binding of scFv to green fluorescent protein fused to the amino-terminal 32-kDa fragment of B. anthracis edema factor, EGFP-EF32, was used to confirm specificity. Fusion of EGFP to PA32 facilitated development of a novel flow cytometric assay that showed that one of the scFv disrupted PA receptor binding. This method can now be used as a rapid assay for small molecule inhibitors of PA binding to cell receptors. The combined data presented suggest the potential utility of human scFv as prophylactics against anthrax poisoning. Moreover, recombinant PA32 may also be useful as a therapeutic agent to compete with anthrax toxins for cellular receptors during active infection.

Descriptors: *Antibodies, Bacterial--immunology--IM; *Antigens, Bacterial --immunology--IM; *Bacterial Toxins--immunology--IM; *Immunoglobulin Fragments--immunology--IM; Animals; Antibody Affinity; Binding, Competitive ; CHO Cells; Cricetinae; Electrophoresis, Polyacrylamide Gel; Flow Cytometry--methods--MT; Humans; Receptors, Peptide--immunology--IM; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Sodium Dodecyl Sulfate; Time Factors

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial); 0 (Bacterial Toxins); 0 (Immunoglobulin Fragments); 0 (Receptors, Peptide); 0 (anthrax toxin); 0 (anthrax toxin receptors); 0 (immunoglobulin Fv); 151-21-3 (Sodium Dodecyl Sulfate)

Record Date Created: 19990628

Record Date Completed: 19990628

10/9/14 (Item 14 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

12347540 PMID: 10103007

Selection-dominant and nonaccessible epitopes on cell-surface receptors revealed by cell-panning with a large phage antibody library.

Hoogenboom H R; Lutgerink J T; Pelsers M M; Rousch M J; Coote J; Van Neer N; De Bruine A; Van Nieuwenhoven F A; Glatz J F; Arends J W

CESAME at the Department of Pathology, Maastricht University, The Netherlands. hho@lpat.azm.nl

European journal of biochemistry / FEBS (GERMANY) Mar 1999, 260 (3) p774-84, ISSN 0014-2956--Print Journal Code: 0107600

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

To generate antibodies to defined cell-surface antigens, we used a large phage antibody fragment library to select on cell transfectants expressing one of three chosen receptors. First, in vitro panning procedures and phage antibody screening ELISAs were developed using whole live cells stably expressing the antigen of interest. When these methodologies were applied to Chinese hamster ovary (CHO) cells expressing one of the receptors for a neuropeptide, somatostatin, using either direct cell panning or a strategy of depletion or ligand-directed elution, many different pan-CHO-cell binders were selected, but none was receptor specific. However, when using direct panning on CHO-cells expressing the human membrane protein CD36, an extraordinary high frequency of antigen-specific phage antibodies was found. Panning on myoblasts expressing the rat homologue of CD36 revealed a similar selection dominance for anti-(CD36). Binding of all selected 20

different anti-(CD36) phage was surprisingly inhibited by one anti-(CD36) mAb CLB-IVC7, which recognizes a functional epitope that is also immunodominant in vivo. Similar inhibition was found for seven anti-(rat) CD36 that cross-reacted with human CD36. Our results show that, although cells can be used as antigen carriers to select and screen phage antibodies, the nature of the antigen target has a profound effect on the outcome of the selection.

Descriptors: *Antigens, CD36--immunology--IM; *Immunodominant Epitopes --immunology--IM; *Receptors, Somatostatin--immunology--IM; Animals; Antibodies, Viral--immunology--IM; Antibody Specificity; Bacteriophage M13 --immunology--IM; CHO Cells; Cricetinae; Humans; Peptide Library; Rats; Research Support, Non-U.S. Gov't

CAS Registry No.: 0 (Antibodies, Viral); 0 (Antigens, CD36); 0 (Immunodominant Epitopes); 0 (Peptide Library); 0 (Receptors, Somatostatin)

Record Date Created: 19990506

Record Date Completed: 19990506

10/9/15 (Item 15 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11989524 PMID: 9822258

Generation of the single chain antibody fragment conserves the idiotypic profile of the anti-CD30 monoclonal antibody HRS3.

Hombach A; Pohl C; Heuser C; Sircar R; Koch D; Diehl V; Abken H

Klinik 1 fur Innere Medizin, Labor Tumorgenetik, Universitat zu Koln, Germany.

Scandinavian journal of immunology (ENGLAND) Nov 1998, 48 (5) p497-501, ISSN 0300-9475--Print Journal Code: 0323767

Publishing Model Print; Erratum in Scand J Immunol 1999 Aug;50(2) 232

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Recombinant single chain antibody fragments (scFv) derived by combining immunoglobulin VL and VH regions provide valuable antibody-like reagents. A number of them are shown to have retained the antigen specificity of the parental monoclonal antibody (MoAb). Little is known about the idiotypic profile of scFv fragments compared with that of the parental MoAb. To address this question we analysed the idiotypic profile of a scFv that was derived by phage-display techniques from the anti-CD30 MoAb HRS3. We assayed (i) binding of HRS3-scFv to recombinant CD30-Fc antigen and to four different anti-idiotypic MoAbs defining at least three different idiotopes on HRS3, and (ii) cross-competition with the parental MoAb HRS3 and the closely related anti-CD30 MoAb HRS4. The assays revealed that the HRS3-scFv fragment exhibits the same specificity for both CD30 antigen and the tested anti-idiotypic MoAbs compared with the parental MoAb demonstrating that the recombinant scFv fragment has retained the complete idiotope of the parental MoAb.

Descriptors: *Antibodies, Monoclonal--immunology--IM; *Antigens, CD30 --immunology--IM; *Immunoglobulin Fragments--immunology--IM; *Immunoglobuli n Idiotypes--immunology--IM; *Immunoglobulin Variable Region--immunology --IM; Animals; CHO Cells; Cricetinae; Humans; Recombinant Fusion Proteins --immunology--IM; Research Support, Non-U.S. Gov't; Tumor Cells, Cultured

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens, CD30); 0 (Immunoglobulin Fragments); 0 (Immunoglobulin Idiotypes); 0 (Immunoglobulin Variable Region); 0 (Recombinant Fusion Proteins)

Record Date Created: 19981211
Record Date Completed: 19981211

10/9/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11317000 PMID: 9129313
Role of high-performance liquid chromatographic protein analysis in developing fermentation processes for recombinant human growth hormone, relaxin, antibody fragments and lymphotoxin.
Jacobson F S; Hanson J T; Wong P Y; Mulkerrin M; Deveney J; Reilly D; Wong S C
Department of Fermentation and Cell Culture Process Development, Genentech, Inc., South San Francisco, CA 94080, USA.
Journal of chromatography. A (NETHERLANDS) Feb 28 1997, 763 (1-2) p31-48, ISSN 0021-9673--Print Journal Code: 9318488
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Development of efficient and reliable fermentation processes for protein pharmaceuticals is aided by the availability of accurate quantitative and qualitative product analyses. We have developed a variety of single and dual column chromatographic separations that meet the needs of process development and examples will be provided of how the resulting data has been used to optimize the culture process. For single column methods, reversed-phase chromatography has been the most versatile, permitting the reliable quantitation of many yeast, Chinese hamster ovary (CHO) cell and Escherichia coli-expressed products in the matrix of culture broth or cell extract. Analysis of secreted human growth hormone synthesized in E. coli, along with clipped and unprocessed forms, will be discussed. Another reversed-phase assay for direct analysis of a peptide product (B-chain relaxin) and its degradation products secreted into E. coli fermentation medium has allowed the purification of the responsible protease. Cation-exchange has proven extremely useful for the direct analysis of antibody fragment synthesized in E. coli, allowing the separation and quantitation of the desired Fab' and Fab'2, as well as the unwanted products of glutathione addition and translational read-through. Assay development is often complicated by the presence of host proteins with chromatographic behavior that is similar to that of the product. Commercial instrumentation now permits the facile development of multidimensional chromatographic assays. We show examples of coupled receptor affinity-reversed-phase assays for a mistranslation product and for covalent multimers of E. coli-synthesized lymphotoxin.
Descriptors: *Chromatography, High Pressure Liquid--methods--MT; *Fermentation; *Human Growth Hormone--biosynthesis--BI; *Immunoglobulin Fragments--biosynthesis--BI; *Lymphotoxin--biosynthesis--BI; *Relaxin --biosynthesis--BI; Amino Acid Sequence; Animals; CHO Cells; Cricetinae; Escherichia coli--metabolism--ME; Human Growth Hormone--analysis--AN; Humans; Immunoglobulin Fragments--analysis--AN; Lymphotoxin--analysis--AN; Recombinant Proteins--analysis--AN; Recombinant Proteins--biosynthesis--BI ; Relaxin--analysis--AN; Sequence Analysis; Technology, Pharmaceutical
CAS Registry No.: 0 (Immunoglobulin Fragments); 0 (Lymphotoxin); 0 (Recombinant Proteins); 12629-01-5 (Human Growth Hormone); 9002-69-1 (Relaxin)
Record Date Created: 19970605

Record Date Completed: 19970605

10/9/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10210480 PMID: 7954464
Improved tumor targeting with chemically cross-linked recombinant antibody fragments.
King D J; Turner A; Farnsworth A P; Adair J R; Owens R J; Pedley R B; Baldock D; Proudfoot K A; Lawson A D; Beeley N R; et al
Celltech Ltd., Berkshire, United Kingdom.
Cancer research (UNITED STATES) Dec 1 1994, 54 (23) p6176-85, ISSN 0008-5472--Print Journal Code: 2984705R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The construction and use of recombinant chimeric and later fully humanized (CDR-grafted) antibodies to tumor-associated antigens has reduced the immune response generated to these antibodies in clinical studies. However, their long circulating half-life is a disadvantage for tumor imaging and therapy. Fragments such as F(ab')2, Fab', Fv and single chain Fv (scFv) offer faster blood clearance but also lower overall tumor doses. We have examined the tumor targeting of several novel fragments produced by chemical cross-linking of Fab' or scFv to dimeric and trimeric species. To facilitate cross-linking of Fab' fragments, a chimeric B72.3 Fab' fragment has been expressed with a hinge sequence containing a single cysteine residue. B72.3 scFv was also produced with a similar hinge region peptide attached to the COOH terminus to allow cross-linking. These fragments, Fab' delta Cys and scFv' delta Cys were cross-linked with linkers containing two or three maleimide groups to produce dimeric and trimeric molecules with increased avidity for antigen. Cross-linkers were also designed to contain a 12-N-4 macrocycle capable of stable radiolabeling with 90Y. This allowed the production of site-specifically-labeled, fully immunoreactive proteins. Biodistribution studies in the nude mouse LS174T xenograft model with scFv, di-scFv, and tri-scFv demonstrated that these fragments clear extremely rapidly from the circulation and give rise to only low levels of activity accumulated at the tumor. Di-Fab (DFM) and tri-Fab (TFM) however, accumulated relatively high levels of activity at the tumor with high tumor:blood ratios generated, demonstrating improved targeting compared to IgG. cB72.3 90Y-labeled tri-Fab was found not to accumulate in the kidney or the bone, resulting in an attractive antibody fragment for tumor therapy.
Descriptors: *Immunoglobulin Fab Fragments--therapeutic use--TU; *Immunoglobulin Fragments--therapeutic use--TU; *Neoplasms, Experimental --radiotherapy--RT; *Radioimmunotherapy; Animals; CHO Cells; Cattle; Cricetinae; Immunoglobulin Fab Fragments--metabolism--ME; Immunoglobulin Fragments--metabolism--ME; Mice; Mice, Nude; Neoplasm Transplantation; Recombinant Proteins--metabolism--ME; Recombinant Proteins--therapeutic use--TU; Research Support, Non-U.S. Gov't; Transplantation, Heterologous; Yttrium Radioisotopes--therapeutic use--TU
CAS Registry No.: 0 (Immunoglobulin Fab Fragments); 0 (Immunoglobulin Fragments); 0 (Recombinant Proteins); 0 (Yttrium Radioisotopes); 0 (immunoglobulin Fv)
Record Date Created: 19941227
Record Date Completed: 19941227

10/9/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09148459 PMID: 1736881
Expression, purification and characterization of a mouse-human chimeric antibody and chimeric Fab' fragment.
King D J; Adair J R; Angal S; Low D C; Proudfoot K A; Lloyd J C; Bodmer M W; Yarranton G T
Celltech Ltd., Berks, U.K.
Biochemical journal (ENGLAND) Jan 15 1992, 281 (Pt 2) p317-23, ISSN 0264-6021--Print Journal Code: 2984726R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
B72.3 is a mouse monoclonal antibody against a tumour-associated antigen, TAG72, which recognizes breast, ovarian and colorectal tumour tissue. A mouse-human chimeric version of B72.3 has been expressed in Chinese-hamster ovary cells. This molecule has the binding specificity of B72.3 and constant regions from human IgG4. The chimeric B72.3 assembles to intact IgG and recognizes TAG72 as well as B72.3 in competitive binding assays. A proportion of the chimeric B72.3 (approx. 10%) does not form inter-heavy-chain disulphide bonds but still assembles into the IgG tetramer. This appears to be a general property of human IgG4 molecules. Co-expression of the chimeric light chain with a chimeric Fd' gene resulted in the expression of functional Fab'. Very little F(ab')2 is produced, although the Fab' can be oxidized to the dimeric F(ab')2 in vitro. The production of Fab' and F(ab')2 by this method is an attractive alternative to proteolytic digestion of IgG. The ability to produce these molecules in large quantities will allow the production and testing of a range of anti-tumour antibody and antibody fragment conjugates.
Descriptors: *Antibodies, Monoclonal--immunology--IM; *Antigens, Neoplasm --immunology--IM; *Immunoglobulin Fab Fragments--immunology--IM; Animals; Antibodies, Monoclonal--genetics--GE; Antibodies, Monoclonal--isolation and purification--IP; CHO Cells; Chimera--immunology--IM; Chromatography, High Pressure Liquid; Cricetinae; Electrophoresis, Polyacrylamide Gel; Gene Expression; Humans; Immunoglobulin Fab Fragments--genetics--GE; Immunoglobulin Fab Fragments--isolation and purification--IP; Mice
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens, Neoplasm); 0 (Immunoglobulin Fab Fragments)
Record Date Created: 19920311
Record Date Completed: 19920311

10/9/19 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015857551 BIOSIS NO.: 200600202946
Over expression of anti-MUC1 single-domain antibody fragments in the yeast Pichia pastoris
AUTHOR: Rahbarizadeh Fatemeh; Rasaee Mohammad J (Reprint); Forouzandeh Mehdi; Allameh Abdol-Amir
AUTHOR ADDRESS: Tarbiat Modarres Univ, Dept Clin Biochem, Fac Med Sci, Tehran, Iran**Iran

AUTHOR E-MAIL ADDRESS: mohammadrasaee@yahoo.com
JOURNAL: Molecular Immunology 43 (5): p426-435 FEB 2006 2006
ISSN: 0161-5890
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The methylotrophic yeast Pichia pastoris has become a highly popular expression host system for the recombinant production of a wide variety of proteins, such as antibody fragments. Camelids produce functional antibodies devoid of light chains and constant heavy-chain domain (CHI). The antigen binding fragments of such heavy chain antibodies are therefore comprised in one single domain, the so-called VH of the camelid heavy chain antibody (VHH). To test the feasibility of expressing VHHs in the yeast. which on account of their small size and anti-en recognition properties would have a major impact on antibody engineering strategies, we constructed two VHH genes encoding the single-domain antibody fragments with specificity for a cancer associated mucin, MUC1. The recombinant strains of the yeast P. pastoris were developed which secrete single-domain antibody fragment to the culture supernatant as a biologically active protein. Supplementation of medium with sorbitol (in pre-induction phase) and casamino acid or EDTA (in induction phase) provided ideal condition of increasing the yield of VHH production compared to culture condition devoid of above recipe. The secreted protein was purified following a 80% ammonium sulfate precipitation step, followed by a affinity chromatography column. The specific activity in enzyme-linked immunosorbant assay (ELISA) of the purified yeast VHH was higher than that of a bacterial periplasmic counterpart. These results reaffirm that the east P. pastoris is a suitable host for high level and correctly folded production of VHH antibody fragments with potential in vivo diagnostic and therapeutic applications. This is the first report of expression of VHH in P. pastoris.

REGISTRY NUMBERS: 60-00-4: EDTA; 50-70-4: sorbitol; 7783-20-2: ammonium sulfate
DESCRIPTORS:
MAJOR CONCEPTS: Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Ascomycetes--Fungi, Plantae; Camelidae--Artiodactyla , Mammalia, Vertebrata, Chordata, Animalia; Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: Pichia pastoris (Ascomycetes)--transgenic; Camelus bactrianus (Camelidae); Camelus dromedarius (Camelidae); CHO cell line (Cricetidae)--Hamster cells; Escherichia coli (Enterobacteriaceae); T-47D cell line (Hominidae)--human breast cancer cells; BT-20 cell line (Hominidae)--human breast cancer cells; HT 29 cell line (Hominidae)--human colon cancer cells; MCF-7 5C cell line (Hominidae)--human breast cancer cells
COMMON TAXONOMIC TERMS: Fungi; Nonvascular Plants; Plants; Artiodactyls; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Bacteria; Eubacteria; Microorganisms; Animals; Chordates; Humans; Mammals; Primates; Vertebrates
CHEMICALS & BIOCHEMICALS: EDTA; antibody; sorbitol; ammonium sulfate; antibody fragments; casamino acid
GENE NAME: Camelus MUC1 gene (Camelidae); Camelus VHH gene (Camelidae)--transgene
METHODS & EQUIPMENT: ELISA--laboratory techniques, immunologic

techniques; affinity chromatography--laboratory techniques, chromatographic techniques
CONCEPT CODES:
02506 Cytology - Animal
02508 Cytology - Human
03502 Genetics - General
03504 Genetics - Plant
03506 Genetics - Animal
03508 Genetics - Human
10060 Biochemistry studies - General
10068 Biochemistry studies - Carbohydrates
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
34502 Immunology - General and methods
BIOSYSTEMATIC CODES:
15100 Ascomycetes
85720 Camelidae
86310 Cricetidae
06702 Enterobacteriaceae
86215 Hominidae

10/9/20 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015408635 BIOSIS NO.: 200510103135
Expression and characterisation of recombinant human CD48 and isolation of a human anti-CD48 monoclonal antibody by phage display
AUTHOR: Wei Jiewei; Chin David Y; Catzel Dallia; Pera Natasha 1; Mahler Stephen M (Reprint)
AUTHOR ADDRESS: Univ New S Wales, Sch Biotechnol and Biomol Sci, Ctr Bioengn, Sydney, NSW 2052, Australia**Australia
AUTHOR E-MAIL ADDRESS: s.mahler@unsw.edu.au
JOURNAL: Journal of Chemical Technology and Biotechnology 80 (7): p782-795 JUL 05 2005
ISSN: 0268-2575
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Human CD48, a membrane-bound, glycosylphosphatidylinositol (GPI)-linked glycoprotein, is a potential tumour target for the treatment of leukaemias and lymphomas. CD48 is expressed on T-and B-cells, however < 5% of CD34(+) progenitor cells express CD48. A truncated, 45kDa soluble form of the full length CD48 was expressed in Chinese hamster ovary (CHO) cells, and was shown to consist of a broad range of charge isoforms, with the most abundant isoforms between pI 4.5 and 5.0. The truncated form of CD48 was shown to bind to antibodies raised against native, GPI-linked CD48 by surface plasmon resonance analysis. A synthetic, human, scFv immunoglobulin gene library was screened against recombinant CD48 by phage display, and an scFv antibody fragment, (designated N2A) was isolated after four rounds of biopanning. N2A was reassembled as a human IgG1 human monoclonal antibody, expressed in CHO cells and the binding of IgG1-N2A to recombinant CD48 was confirmed by surface plasmon resonance. Flow cytometry studies of IgG1-N2A binding to Raji cells showed the specificity of N2A for GPI-linked CD48 was conserved, and presents the potential for IgG1-N2A as a lead antibody candidate for the treatment of white blood cell malignancies. (c) 2005 Society of Chemical Industry.

DESCRIPTORS:
MAJOR CONCEPTS: Methods and Techniques; Immune System--Chemical Coordination and Homeostasis
BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: CHO cell line (Cricetidae); human (Hominidae); Raji cell line (Hominidae)
ORGANISMS: PARTS ETC: B cell--immune system, blood and lymphatics; white blood cell--immune system, blood and lymphatics; CD34-positive progenitor cell--immune system
COMMON TAXONOMIC TERMS: Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Animals; Chordates; Humans; Mammals; Primates; Vertebrates
CHEMICALS & BIOCHEMICALS: monoclonal antibody; glycoprotein; glycosylphosphatidylinositol; IgG1 {immunoglobulin G1}; CD48; scFv; IgG1-N2A
METHODS & EQUIPMENT: flow cytometry--laboratory techniques, histology and cytology techniques; surface plasmon resonance--laboratory techniques, spectrum analysis techniques
CONCEPT CODES:
02506 Cytology - Animal
02508 Cytology - Human
10064 Biochemistry studies - Proteins, peptides and amino acids
10066 Biochemistry studies - Lipids
15002 Blood - Blood and lymph studies
15004 Blood - Blood cell studies
34502 Immunology - General and methods
BIOSYSTEMATIC CODES:
86310 Cricetidae
86215 Hominidae

10/9/21 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015144462 BIOSIS NO.: 200500051527
Single-chain antibodies for the conformation-specific blockade of activated platelet integrin alphaIIbbeta3 designed by subtractive selection from naive human phage libraries
AUTHOR: Schwarz Meike (Reprint); Roettgen Peter; Takada Yoshiazuku; Le Gall Fabrice; Knackmuss Stefan; Bassler Nicole; Buttner Claudia; Little Melvyn ; Bode Christoph; Peter Karlheinz
AUTHOR ADDRESS: Dept Cardiol, Univ Freiburg, Breisacherstr 33, D-79106, Freiburg, Germany**Germany
AUTHOR E-MAIL ADDRESS: schwarz@med1.ukl.uni-freiburg.de
JOURNAL: FASEB Journal 18 (12): September 2004 2004
MEDIUM: print
ISSN: 0892-6638 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Binding of fibrinogen to platelet integrin alphaIIbeta3 mediates platelet aggregation, and thus inhibition of alphaIIbbeta3 represents a powerful therapeutic strategy in cardiovascular medicine. However, the currently used inhibitors of aIIbbeta3 demonstrate several adverse effects like thrombocytopenia and bleeding, which are associated with their property to bind to non-activated alphaIIbbeta3. To circumvent these problems, we designed blocking single-chain antibody-fragments

(scFv) that bind to alphaIIbbeta3 exclusively in its activated conformation. Two nave phage libraries were created: a natural phage library, based on human lymphocyte cDNA, and a synthetic library, with randomized VHCDR3. We performed serial rounds of subtractive panning with depletion on non-activated and selection on activated aIIbbeta3, which were provided on resting and ADP-stimulated platelets and CHO cells, expressing wild-type or mutated and thereby activated alphaIIbbeta3. In contrast to isolated, immobilized targets, as generally used for phage display, this unique cell-based approach for panning allowed the preservation of functional integrin conformation. Thereby, we obtained several scFv-clones that demonstrated exclusive binding to activated platelets and complete inhibition of fibrinogen binding and platelet aggregation. Interestingly, all activation-specific clones contained an RXD pattern in the HCDR3. Binding studies on transiently expressed point mutants and mouse-human domain-switch mutants of alphaIIbbeta3 indicate a binding site similar to fibrinogen. In conclusion, we generated human activation-specific scFvs against alphaIIbbeta3, which bind selectively to activated alphaIIbbeta3 and thereby potently inhibit fibrinogen binding to alphaIIbbeta3 and platelet aggregation.

REGISTRY NUMBERS: 58-64-0Q: ADP; 4792-83-0Q: ADP; 7722-76-1Q: ADP; 19429-39-1Q: ADP; 175832-20-9Q: ADP
DESCRIPTORS:
MAJOR CONCEPTS: Cardiovascular Medicine--Human Medicine, Medical Sciences ; Cell Biology; Clinical Immunology--Human Medicine, Medical Sciences; Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata , Animalia; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: CHO cell line (Cricetidae); human (Hominidae)
ORGANISMS: PARTS ETC: lymphocyte--blood and lymphatics, immune system; platelet--blood and lymphatics
COMMON TAXONOMIC TERMS: Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Animals; Chordates; Humans; Mammals; Primates; Vertebrates
DISEASES: thrombocytopenia--blood and lymphatic disease
MESH TERMS: Thrombocytopenia (MeSH)
CHEMICALS & BIOCHEMICALS: ADP; complementary DNA {cDNA}; fibrinogen; integrin alpha-11b-beta-3; scFv antibody; single-chain antibody-fragment
MISCELLANEOUS TERMS: phage library; phage-display
CONCEPT CODES:
02502 Cytology - General
02506 Cytology - Animal
02508 Cytology - Human
03502 Genetics - General
03506 Genetics - Animal
03508 Genetics - Human
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10064 Biochemistry studies - Proteins, peptides and amino acids
14506 Cardiovascular system - Heart pathology
15002 Blood - Blood and lymph studies
15004 Blood - Blood cell studies
15006 Blood - Blood, lymphatic and reticuloendothelial pathologies
34502 Immunology - General and methods
34508 Immunology - Immunopathology, tissue immunology
BIOSYSTEMATIC CODES:
86310 Cricetidae
86215 Hominidae

10/9/22 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015070405 BIOSIS NO.: 200400438324
Disruption of P-glycoprotein anticancer drug efflux activity by a small recombinant single-chain Fv antibody fragment targeted to an extracellular epitope
AUTHOR: Haus-Cohen Maya; Assaraf Yehuda G; Binyamin Liat; Benhar Itai; Reiter Yoram (Reprint)
AUTHOR ADDRESS: Dept Biol, Technion Israel Inst Technol, IL-32000, Haifa, Israel**Israel
AUTHOR E-MAIL ADDRESS: reiter@tx.technion.ac.il
JOURNAL: International Journal of Cancer 109 (5): p750-758 May 1, 2004 2004
MEDIUM: print
ISSN: 0020-7136
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Inherent and acquired MDR is characterized by simultaneous resistance to diverse anticancer drugs and continues to be a major impediment in the curative chemotherapy of cancer. The MDR I gene product, Pgp, is an ATP-driven efflux pump, which extrudes a variety of dissimilar hydrophobic cytotoxic compounds from MDR cells. Pgp overexpression results in MDR of tumor cell lines in vitro as well as of a variety of human malignancies. Thus, one major goal is to develop strategies aimed at specifically disrupting Pgp drug-efflux activity. To this end, we have developed a small recombinant antibody capable of potent reversal of MDR, by disrupting Pgp drug-efflux activity. Using a phage display approach, we isolated a small scFv recombinant antibody fragment that specifically reacts with the first extracellular loop of human Pgp. This scFv fragment binds specifically to various Pgp-overexpressing human MDR carcinoma cell lines, consequently disrupts Pgp drug-efflux function and thereby reverses the MDR phenotype. We have successfully disrupted anticancer drug-extrusion pump activity in MDR cells using a small recombinant scFv fragment. We propose that these novel small Fv-based recombinant antibody molecules may lead to the development of a new class of antibody fragment-based agents that specifically inhibit Pgp drug extrusion. Hence, these small recombinant antibody fragments may be applied in combination chemotherapy to overcome MDR in various human cancers. Copyright 2004 Wiley-Liss, Inc.

DESCRIPTORS:
MAJOR CONCEPTS: Molecular Genetics--Biochemistry and Molecular Biophysics ; Oncology--Human Medicine, Medical Sciences; Pharmaceuticals--Pharmacology
BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata , Animalia; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: CHO cell line (Cricetidae); human (Hominidae)
COMMON TAXONOMIC TERMS: Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Animals; Chordates; Humans; Mammals; Primates; Vertebrates
DISEASES: cancer--neoplastic disease, diagnosis, drug therapy, epidemiology, etiology, genetics, pathology, prevention and control , radiotherapy, symptom, therapy
MESH TERMS: Neoplasms (MeSH)
CHEMICALS & BIOCHEMICALS: P-glycoprotein {Pgp}--ATP-driven efflux pump, anticancer agent, expression, extracellular loop, role; cytotoxic

compounds--extrusion, hydrophobic; single-chain Fv antibody fragment {scFv antibody fragment}--isolation, role
GENE NAME: human MDR1 gene (Hominidae)
METHODS & EQUIPMENT: combination chemotherapy--clinical techniques, therapeutic and prophylactic techniques; phage display--genetic techniques, laboratory techniques; small Fv-based recombinant antibody molecules--laboratory equipment
MISCELLANEOUS TERMS: P-glycoprotein drug-efflux activity {Pgp drug-efflux activity}--disruption; multidrug resistance {MDR}--acquired, inherent, phenotype
CONCEPT CODES:
03502 Genetics - General
03506 Genetics - Animal
03508 Genetics - Human
10064 Biochemistry studies - Proteins, peptides and amino acids
10068 Biochemistry studies - Carbohydrates
12502 Pathology - General
12504 Pathology - Diagnostic
12512 Pathology - Therapy
22002 Pharmacology - General
24001 Neoplasms - Diagnostic methods
24004 Neoplasms - Pathology, clinical aspects and systemic effects
24008 Neoplasms - Therapeutic agents and therapy
37054 Public health: epidemiology - Organic diseases and neoplasms
37056 Public health: epidemiology - Miscellaneous
BIOSYSTEMATIC CODES:
86310 Cricetidae
86215 Hominidae

10/9/23 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0014382625 BIOSIS NO.: 200300339368
Nucleocytoplasmic shuttling of antigen in mammalian cells conferred by a soluble versus insoluble single-chain antibody fragment equipped with import/export signals.
AUTHOR: Sibler Annie-Paule; Nordhammer Alexandra; Masson Murielle; Martineau Pierre; Trave Gilles; Weiss Etienne (Reprint)
AUTHOR ADDRESS: Biotechnologie des Interactions Macromoleculaires, UMR 7100, Ecole Superieure de Biotechnologie de Strasbourg, boulevard Sebastien Brant, 67400, Illkirch, France**France
AUTHOR E-MAIL ADDRESS: eweiss@esbs.u-strasbg.fr
JOURNAL: Experimental Cell Research 286 (2): p276-287 June 10, 2003 2003
MEDIUM: print
ISSN: 0014-4827 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The ectopic expression of antibody fragments within mammalian cells is a challenging approach for interfering with or even blocking the biological function of the intracellular target. For this purpose, single-chain Fv (scFv) fragments are generally preferred. Here, by transfecting several mammalian cell lines, we compared the intracellular behavior of two scFvs (13R4 and 1F4) that strongly differ in their requirement of disulphide bonding for the formation of active molecules in bacteria. The scFv 13R4, which is correctly folded in the bacterial cytoplasm, was solubly expressed in all cell lines tested and was

distributed in their cytoplasm and nucleus, as well. In addition, by appending to the 13R4 molecules the SV40 T-antigen nuclear localisation signal (NLS) tag, cytoplasmic-coexpressed antigen was efficiently retargeted to the nucleus. Compared to the scFv 13R4, the scFv 1F4, which needs to be secreted in bacteria for activity, accumulated, even with the NLS tag, as insoluble aggregates within the cytoplasm of the transfected cells, thereby severely disturbing fundamental functions of cell physiology. Furthermore, by replacing the NLS tag with a leucine-rich nuclear export signal (NES), the scFv 13R4 was exclusively located in the cytoplasm, whereas the similarly modified scFv 1F4 still promoted cell death. Coexpression of NES-tagged 13R4 fragments with nuclear antigen promoted its efficient retargeting to the cytoplasm. This dominant effect of the NES tag was also observed after exchange of the nuclear signals between the scFv 13R4 and its antigen. Taken together, the results indicate that scFvs that are active in the cytoplasm of bacteria may behave similarly in mammalian cells and that the requirement of their conserved disulphide bridges for activity is a limiting factor for mediating the nuclear import/export of target in a mammalian cell context. The described shuttling effect of antigen conferred by a soluble scFv may represent the basis of a reliable in vivo assay of effective protein- protein interactions.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology; Immune System--Chemical Coordination and Homeostasis
BIOSYSTEMATIC NAMES: Mammalia--Vertebrata, Chordata, Animalia; Cercopithecidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: mammal (Mammalia); COS1 cell line (Cercopithecidae)--African green monkey kidney cells; HeLa cell line (Hominidae)--human cells; CHO cell line (Cricetidae)--Chinese hamster ovary cells
COMMON TAXONOMIC TERMS: Nonhuman Primates; Humans; Primates; Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates
CHEMICALS & BIOCHEMICALS: single-chain antibody fragment--import/export signal; SV40 T-antigen--nuclear localization signal, leucine-rich nuclear export signal; soluble antibody fragment
CONCEPT CODES:
02502 Cytology - General
02506 Cytology - Animal
02508 Cytology - Human
10060 Biochemistry studies - General
34502 Immunology - General and methods
BIOSYSTEMATIC CODES:
85700 Mammalia
86205 Cercopithecidae
86215 Hominidae
86310 Cricetidae

10/9/24 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0014382058 BIOSIS NO.: 200300338801
High-yield production of recombinant antibody fragments in HEK-293 cells using sodium butyrate.
AUTHOR: Grunberg Jurgen (Reprint); Knogler Karin; Waibel Robert; Novak-Hofer Ilse

AUTHOR ADDRESS: Center for Radiopharmaceutical Science, Paul Scherrer Institute, CH-5232, Villigen, Switzerland**Switzerland
AUTHOR E-MAIL ADDRESS: juergen.gruenberg@psi.ch
JOURNAL: BioTechniques 34 (5): p968-972 May 2003 2003
MEDIUM: print
ISSN: 0736-6205 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To develop new recombinant monoclonal antibody fragments for therapy and imaging, it is indispensable to have a simple and easy procedure to handle the eukaryotic expression system for production of proteins in high amounts. Gene amplification techniques such as the dehydrofolate reductase (DHFR) system in Chinese hamster ovary cells or the glutamine synthase system in myeloma cells have a couple of disadvantages. The selection procedure is complex, time-consuming, and not fruitful in all cases. The toxic drug methotrexate (for the DHFR system) can increase the production rate but decreases the specific growth rate of the cells. The production rate is not always stable over a long-term cultivation period. To overcome these problems, we are using stably transfected human embryonic kidney (HEK-293) cells in combination with an efficient screening method. Sodium butyrate can increase the expression of recombinant antibody fragments in the transfectomas up to 500 mug/4.2X107 cells/24 h corresponding to 175 mu/mL culture medium. This strategy allows a rapid development of new recombinant monoclonal antibody fragments and allows one to proceed rapidly to in vivo testing.

REGISTRY NUMBERS: 156-54-7: sodium butyrate
DESCRIPTORS:
MAJOR CONCEPTS: Cell Biology; Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: HEK-293 cell line (Hominidae)--human embryonic kidney cells; CHO cell line (Cricetidae)--Chinese hamster ovary cells
COMMON TAXONOMIC TERMS: Humans; Primates; Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates
CHEMICALS & BIOCHEMICALS: recombinant antibody fragment; sodium butyrate; recombinant antibody fragments
METHODS & EQUIPMENT: gene amplification--genetic techniques, laboratory techniques
MISCELLANEOUS TERMS: high-yield production
CONCEPT CODES:
02502 Cytology - General
02506 Cytology - Animal
02508 Cytology - Human
03502 Genetics - General
03506 Genetics - Animal
03508 Genetics - Human
BIOSYSTEMATIC CODES:
86215 Hominidae
86310 Cricetidae

10/9/25 (Item 7 from file: 5)

0013140683 BIOSIS NO.: 200100312522

Engineering measles virus as an oncolytic agent for the treatment of multiple myeloma

AUTHOR: Peng Kah-Whye (Reprint); Donovan Kathleen (Reprint); Zhang Jie (Reprint); Schneider Urs (Reprint); Lust John (Reprint); Cattaneo Roberto (Reprint); Russell Stephen J (Reprint)
AUTHOR ADDRESS: Mayo Foundation, Rochester, MN, USA**USA

JOURNAL: Blood 96 (11 Part 1): p512a November 16, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000; 20001201
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A variety of oncolytic viruses have shown promise for tumor therapy. Ideally, the virus should be potently toxic to target cells but cause limited damage to normal tissues. We demonstrate here that a previously unexplored agent, the Edmonston B vaccine strain of measles virus (MV-Edm), has anti-neoplastic activity against large human multiple myeloma xenografts and that the virus can be engineered to enter and spread in susceptible cells in a receptor dependent way. MV-Edm infected and replicated efficiently in ARH-77 and RPMI 8226 myeloma cell lines, causing massive cell-cell fusion and formation of multinucleated syncytia. On non-transformed lymphocytes, the replicative spread of the virus was at least 100-fold less efficient. When administered intratumorally into established multiple myeloma xenografts in immunocompromised mice, MV-Edm repressed the growth of all treated tumors. MV-Edm was most potent against the ARH-77 myeloma xenografts. When delivered intravenously as a single dose or multiple doses, MV-Edm caused complete regression of all of the ARH-77 myeloma xenografts. To generate an engineered MV suitable for targeted myeloma therapy, a single chain antibody fragment (scFv) to a myeloma cell surface antigen, CD38, was generated. A scFv generated against CD52, a lymphocyte antigen, was generated as a control. The respective scFvs were fused to the C-terminus of the MV-H glycoprotein. The recombinant viruses replicated as efficiently as the standard virus on Vero African green monkey kidney cells indicating that they retained their ability to mediate infection through CD46 (natural receptor for measles virus). The targeting properties of the recombinant viruses were tested on parental CHO cells (CD46 negative) or CD38-expressing CHO cells. Standard and alpha-CD52 MV viruses were unable to infect CHO or CD38-CHO cells. The alpha-CD38 MV infected and replicated efficiently in CD38-CHO cells inducing cell-cell fusion and cell death. Infectivity on the CD38-CHO cells was ablated by proteolytic removal of the scFv. MV-Edm merits further study as a novel therapeutic agent for the treatment of multiple myeloma.

DESCRIPTORS:
MAJOR CONCEPTS: Immune System--Chemical Coordination and Homeostasis; Blood and Lymphatics--Transport and Circulation; Tumor Biology
BIOSYSTEMATIC NAMES: Cercopithecidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Paramyxoviridae--Negative Sense ssRNA Viruses, Viruses, Microorganisms
ORGANISMS: Vero cell line (Cercopithecidae)--African green monkey kidney cells; CHO cell line (Cricetidae)--Chinese hamster ovary cells; ARH-77

cell line (Hominidae)--human myeloma cells; RPMI 8226 cell line (Hominidae)--human myeloma cells; mouse (Muridae)--immunocompromised; measles virus {MV-Edm} (Paramyxoviridae)--intratumoral administration, intravenous administration, oncolytic agent, strain-Edmonton B vaccine
ORGANISMS: PARTS ETC: lymphocytes--blood and lymphatics, immune system; multinucleated syncytia
COMMON TAXONOMIC TERMS: Nonhuman Primates; Humans; Primates; Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates; Microorganisms; Negative Sense Single-Stranded RNA Viruses ; Viruses
DISEASES: multiple myeloma--blood and lymphatic disease, immune system disease, neoplastic disease, treatment
MESH TERMS: Multiple Myeloma (MeSH)
CHEMICALS & BIOCHEMICALS: CD38--antigen; CD46; CD52--antigen; glycoprotein; single chain antibody fragment {scFv}
MISCELLANEOUS TERMS: cell death; cell-cell fusion; Meeting Abstract; Meeting Abstract
CONCEPT CODES:
15006 Blood - Blood, lymphatic and reticuloendothelial pathologies
00520 General biology - Symposia, transactions and proceedings
02506 Cytology - Animal
02508 Cytology - Human
10064 Biochemistry studies - Proteins, peptides and amino acids
15002 Blood - Blood and lymph studies
15004 Blood - Blood cell studies
24003 Neoplasms - Immunology
24004 Neoplasms - Pathology, clinical aspects and systemic effects
24008 Neoplasms - Therapeutic agents and therapy
24010 Neoplasms - Blood and reticuloendothelial neoplasms
33506 Virology - Animal host viruses
34502 Immunology - General and methods
34508 Immunology - Immunopathology, tissue immunology
BIOSYSTEMATIC CODES:
86205 Cercopithecidae
86310 Cricetidae
86215 Hominidae
86375 Muridae
03503 Paramyxoviridae

10/9/26 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0006362311 BIOSIS NO.: 198936071202
EXPRESSION OF FC RECEPTORS IN COMPLEMENTARY DNA TRANSFECTED CHO CELLS ASSAYED IN HV-UHR-SEM BY LABELLING WITH INDIVIDUAL FAB MOLECULES DERIVED FROM MONOCLONAL ANTIBODIES
AUTHOR: PETERS K-R (Reprint); MIETTINEN H; MELLMAN I
AUTHOR ADDRESS: DEP CELL BIOL, YALE UNIV, NEW HAVEN, CONN, USA**USA
JOURNAL: Journal of Cell Biology 107 (6 PART 3): p67A 1988
CONFERENCE/MEETING: JOINT MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY AND THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, SAN FRANCISCO, CALIFORNIA, USA, JANUARY 29-FEBRUARY 2, 1989. J CELL BIOL.
ISSN: 0021-9525
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
DESCRIPTORS: ABSTRACT CHINESE HAMSTER MEMBRANE PROTEIN ANTIBODY FRAGMENT

HIGH VOLTAGE-ULTRA HIGH RESOLUTION SCANNING ELECTRON MICROSCOPY
DESCRIPTORS:
  MAJOR CONCEPTS: Cell Biology; Genetics; Immune System--Chemical Coordination and Homeostasis; Membranes--Cell Biology; Methods and Techniques
  BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia
  COMMON TAXONOMIC TERMS: Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates
CONCEPT CODES:
  00520 General biology - Symposia, transactions and proceedings
  01054 Microscopy - Cytology and cytochemistry
  01058 Microscopy - Electron microscopy
  02506 Cytology - Animal
  03506 Genetics - Animal
  10054 Biochemistry methods - Proteins, peptides and amino acids
  10064 Biochemistry studies - Proteins, peptides and amino acids
  10068 Biochemistry studies - Carbohydrates
  10508 Biophysics - Membrane phenomena
  34502 Immunology - General and methods
  34508 Immunology - Immunopathology, tissue immunology
BIOSYSTEMATIC CODES:
  86310 Cricetidae

10/9/27 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

12217567 EMBASE No: 2003330343
Adhesion of monocytes to medical steel as used for vascular stents is mediated by the integrin receptor Mac-1 (CD11b/CD18; alphaSUBM betaSUB2) and can be inhibited by semiconductor coating
Schuler P.; Assefa D.; Ylanne J.; Basler N.; Olschewski M.; Ahrens I.; Nordt T.; Bode C.; Peter K.
Dr. K. Peter, Department of Cardiology/Angiology, University of Freiburg, Hugstetter Str. 55, 79106 Freiburg Germany
AUTHOR EMAIL: peterkh@medizin.ukl.uni-freiburg.de
Cell Communication and Adhesion (CELL COMMUN. ADHES.) (United States) 2003, 10/1 (17-26)
CODEN: CCAEB ISSN: 1541-9061
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 32

Implantation of stents into stenosed arteries helps to restore normal blood flow in ischemic organs. However, limited biocompatibility of the applied medical steel can cause acute thrombosis and long-term restenosis. Adhesion of monocytes to stent metal may participate in those acute and long-term complications of stent placement. Based on described prominent electrochemical properties of the interaction between the monocyte integrin receptor Mac-1 and its various ligands, we hypothesized, that this receptor is a central mediator of monocyte adhesion to stent metal and that semiconductor coating of medical steel reduces monocyte adhesion. Adhesion of monocytes on L-316 stainless steel was directly evaluated by light microscopy. Mac-1 could be identified as mediator of monocyte adhesion, since cell adhesion could be blocked by anti-Mac-1-antibodies, including the cross-reacting anti-GPIIb/IIIa antibody fragment abciximab. To further prove the central role of Mac-1, two CHO cell lines were generated expressing recombinant Mac-1 either as wild type, resulting in a low

affinity receptor, or mutant with a GFFKR deletion of the alphaSUBM subunit, resulting in a high affinity receptor. Indeed, adhesion was specific for Mac-1 and dependent on the affinity state of this integrin. Finally, we could demonstrate that Mac-1-mediated adhesion of monocytes to stents can be significantly inhibited by silicon carbide coating of the stent metal. In conclusion, the integrin Mac-1 and its affinity state could be identified as major mediators of monocyte adhesion on medical steel. As therapeutic strategies, the blockade of Mac-1 by antibodies or silicon carbide coating of steel inhibits monocyte adhesion on stents.

BRAND NAME/MANUFACTURER NAME: reopro/Lilly/United States; aspirin
MANUFACTURER NAMES: Lilly/United States
DEVICE BRAND NAME/MANUFACTURER NAME: Tenax/Biotronik/Germany
DEVICE MANUFACTURER NAMES: Biotronik/Germany
DRUG DESCRIPTORS:
*stainless steel; *integrin receptor--endogenous compound--ec
CD11b antigen--endogenous compound--ec; CD18 antigen--endogenous compound --ec; metal; ligand--endogenous compound--ec; receptor antibody --pharmacology--pd; abciximab--pharmacology--pd; recombinant receptor --endogenous compound--ec; receptor subunit--endogenous compound--ec; integrin--endogenous compound--ec; silicon carbide; acetylsalicylic acid; pyridine derivative; unclassified drug
MEDICAL DESCRIPTORS:
*stent; *monocyte; *leukocyte adherence
biocompatibility; cell adhesion; material coating; implantation; artery occlusion; artery blood flow; ischemia--surgery--su; device; thrombosis --complication--co; thrombosis--etiology--et; thrombosis--prevention--pc; restenosis--complication--co; restenosis--etiology--et; restenosis --prevention--pc; electrochemical analysis; molecular interaction; microscopy; protein analysis; cross reaction; CHO cell; gene expression; wild type; binding affinity; gene mutation; gene deletion; semiconductor; human; nonhuman; normal human; controlled study; human cell; animal cell; article; priority journal
DRUG TERMS (UNCONTROLLED): integrin receptor Mac 1--endogenous compound--ec
CAS REGISTRY NO.: 12597-68-1 (stainless steel); 143653-53-6 (abciximab); 409-21-2 (silicon carbide); 493-53-8, 50-78-2, 53663-74-4, 53664-49-6, 63781-77-1 (acetylsalicylic acid)
SECTION HEADINGS:
009 Surgery
025 Hematology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical and Experimental Biochemistry
037 Drug Literature Index

10/9/28 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06079299 Genuine Article#: XT933 Number of References: 32
Title: Structure determination of N-linked oligosaccharides engineered at the CH1 domain of humanized LL2
Author(s): Qu ZX; Sharkey RM; Hansen HJ; Goldenberg DM; Leung S (REPRINT)
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Journal: GLYCOBIOLOGY, 1997, V7, N6 (SEP), P803-809
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Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: Two humanized antibody mutants, hLL2HCN1 and hLL2HCN5, engineered with CH1 domain-appended carbohydrates (CHOs) were generated to facilitate site-specific conjugation of radionuclides and anti-cancer drugs to antibodies, Such site-specific conjugation may minimize the incidence of immunoreactivity perturbation as is often observed with random conjugation, Since the compositions and structures of CHOs are important in determining the chemistry, efficiency, and extent of conjugation, the sequences of the CH1-appended CHOs were determined by exoglycosidase digestions and fluorophore-assisted CHO electrophoresis (FACE), The CHO species attached at HCN1 and HCN5 sites in hLL2HCN1 and hLL2HCN5, respectively, were distinct from each other, heterogeneous, and extensively processed, All of these CHOs were core-fucosylated complex-type oligosaccharides and contained Gal (galactose) and GlcNAc (N-acetylglucosamine) residues in the outer branches, Some of the outer branches were composed of Gal alpha 1-3Gal beta 1-4GlcNAc structure, also known as alpha-galactosyl epitope. Most of the CHOs were sialylated, While all HCN1-CHOs were biantennary, the majority of HCN5-CHOs (>60%) were triantennary, The CH1-appended CHOs have favorable structural characteristics suitable for site-specific conjugation, For efficient conjugation of large drug complexes, hLL2HCN5 is preferable to hLL2HCN1 because the attached CHO is larger in size and more remotely positioned from the V region, The effects of the a-galactosyl epitope found in these CHOs on the immunological properties of the immunoconjugates as efficient cancer diagnostics and therapeutics are being studied.
Descriptors--Author Keywords: cancer ; humanized monoclonal antibody ; immunoconjugates ; oligosaccharides ; site-specific conjugation
Identifiers--KeyWord Plus(R): IMMUNOGLOBULIN-G; MONOCLONAL-ANTIBODIES; SUGAR CHAINS; CARBOHYDRATE; GLYCOSYLATION; SITE; CONJUGATION; IDENTIFICATION; BIOSYNTHESIS; SPECIFICITY
Research Fronts: 95-6231 001 (TC-99M-LABELED LL2 MONOCLONAL-ANTIBODY FRAGMENT; PHASE-I RADIOIMMUNOTHERAPY TRIAL; B-CELL NON-HODGKINS-LYMPHOMA; HIGH-DOSE THERAPY; TUMOR IMAGING)
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S3      38400   (PEPSIN OR (ASPARTYL (W) PROTEASE))
S4          0   S2 AND S3
S5       4495   (PROTEASE (W) (DIGEST OR DIGESTION))
S6          0   S2 AND S5
S7          5   (ANTIBODY (W) FRAGMENT) AND (PROTEASE (W) DIGESTION)
S8          0   (ANTIBODY (W) FRAGMENT) AND (ENZYME (W) DIGESTION)
S9          2   RD S7  (unique items)
S10        28   RD S2  (unique items)
S11         1   S10 AND PROTEASE
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=> s (antibody fragment) and CHO and pepsin
L1            0 (ANTIBODY FRAGMENT) AND CHO AND PEPSIN

=> s (antibody fragment) and cho
L2          681 (ANTIBODY FRAGMENT) AND CHO

=> s (aspartyl protease) or (aspartyl enzyme)
L3         1487 (ASPARTYL PROTEASE) OR (ASPARTYL ENZYME)

=> s l2 and l3
L4            2 L2 AND L3

=> duplicate remove l4
DUPLICATE PREFERENCE IS 'CAPLUS, BIOTECHDS'
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PROCESSING COMPLETED FOR L4
L5             1 DUPLICATE REMOVE L4 (1 DUPLICATE REMOVED)

=> d l5 bib abs 1

L5   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2006 ACS on STN  DUPLICATE 1
AN   2004:354964  CAPLUS
DN   140:373903
TI   Method for cleaving antibodies and generating antibody
     fragments
IN   Zapata, Gerardo A.
PA   Abgenix, Inc., USA
SO   PCT Int. Appl., 21 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
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PI   WO 2004035608        A2    20040429    WO 2003-US33121         20031017
     WO 2004035608        A3    20040812
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             GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
             LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ,
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        RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
            KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
            FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
            BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
    AU 2003286493        A1    20040504    AU 2003-286493         20031017
    US 2004138428        A1    20040715    US 2003-688198         20031017
PRAI US 2002-419908P     P     20021018
    WO 2003-US33121      W     20031017
AB  The disclosed invention is related to a method for producing
    antibody fragments. In particular, the invention
    involves a method for the production of F(ab')2 fragments. The method
    comprises concentration of cell culture media and activation of endogenous
    enzymes present in the cell culture media by adjusting the temperature and pH.
    Cell cultures expressing Igs can be selected from hybridoma or CHO
    cell cultures. For the production of the antibody cells are grown in stirred
    tank bioreactors with appropriate media. Cell culture supernatant is
    harvested and filtered prior to being subjected to pH treatment and
    activation of enzymic cleavage. Enzymes responsible for the enzymic
    activity in the activated cell culture media are aspartyl and cysteinyl
    proteases. For purification of the F(ab')2 fragments generated in the
    digestion reaction, the cell culture media are further subjected to
    several chromatog. steps including a Q Sepharose FF column, a protein A
    column, and a hydrophobic interaction chromatog. (HIC) column.


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    (FILE 'HOME' ENTERED AT 11:05:41 ON 19 JUN 2006)

    FILE 'CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ESBIOBASE' ENTERED AT
    11:06:24 ON 19 JUN 2006
L1            0 S (ANTIBODY FRAGMENT) AND CHO AND PEPSIN
L2          681 S (ANTIBODY FRAGMENT) AND CHO
L3         1487 S (ASPARTYL PROTEASE) OR (ASPARTYL ENZYME)
L4            2 S L2 AND L3
L5            1 DUPLICATE REMOVE L4 (1 DUPLICATE REMOVED)

=> s (enzyme digestion) or (protease digestion)
L6         8803 (ENZYME DIGESTION) OR (PROTEASE DIGESTION)

=> s l2 and l6
L7            1 L2 AND L6

=> d l7 bib abs 1

L7   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN   2003-09763  BIOTECHDS
TI   Optimizing monoclonal antibody expression level, comprises producing a
     host cell having DNA sequences encoding antibody heavy and light chains,
     with an amplifiable marker, culturing the host, and amplifying sequences;
        hybridoma cell culture and plasmid expression in Escherichia coli
AU   WOOD C R; KAUFMAN R J
PA   WYETH
PI   US 6475787 5 Nov 2002
AI   US 1991-765281 25 Sep 1991
PRAI US 1991-765281 25 Sep 1991; US 1989-386489 28 Jul 1989
DT   Patent
LA   English
OS   WPI: 2003-208838 [20]
AN   2003-09763  BIOTECHDS
AB   DERWENT ABSTRACT:
     NOVELTY - Optimizing (M) a monoclonal antibody expression level, by: (a)
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producing a eukaryotic host cell (HC) capable of expressing 2 DNA sequences (DSs) encoding antibody heavy and light chains, where the DSs are associated with heterologous selectable amplifiable marker genes; (b) culturing HC; (c) measuring the relative amounts of DSs expressed; and (d) differentially amplifying DSs with appropriate selective agents.

DETAILED DESCRIPTION - Optimizing (M) the expression level of a monoclonal antibody, comprises: (a) producing a eukaryotic host cell containing and capable of expressing two DNA sequences encoding an antibody heavy and light chain, respectively, where the two DNA sequences are associated with two heterologous selectable amplifiable marker genes, respectively; (b) culturing the host cell in a suitable culture medium; (c) measuring the relative amounts of the two DNA sequences expressed; and (d) differentially amplifying the amounts of the 2 DNA sequences with appropriate selective agents to allow maximized production of the antibody. An INDEPENDENT CLAIM is also included for a host cell (I) produced by (M).

USE - (M) is useful for optimizing the expression level of a monoclonal antibody (claimed). (M) is useful for producing monoclonal antibodies. (M) is useful for the production of cloned antibodies, genetically engineered antibodies such as complementarity determining region (CDR)-swapped antibodies, genetically engineered **antibody fragments** or derivatives, and hetero-dimeric and complete antibodies.

ADVANTAGE - (M) is an improved method for production of monoclonal antibodies.

EXAMPLE - Heavy and light chain cDNAs were cloned from the B1-8 hybridoma cell line (a fusion of a mouse splenocyte and a murine myeloma cell line which produces an immunoglobulin (Ig)M antibody directed to the hapten, 4-hydroxy-3-nitrophenyl acetate). The mu chain DNA and the lambda A chain DNA was each isolated as restriction fragments. The mu chain cDNA was cloned into plasmid pMT3SVA as follows to produce pMT3Amu. The heavy chain expression plasmid was constructed with the p heavy chain cDNA of pABmu-11. The mu cDNA was isolated and prepared for cloning into the EcoRI site of the expression vector pMT35SVA as follows. pABmu-11 was digested to completion with BglII, and then a partial PstI digestion was performed. One resulting BglII-PstI fragment of approximately 1 kb containing the complete 3' end of the cDNA was purified from a low-melt agarose gel. This was then ligated into BamHI and PstI digested Bluescript plasmid and transformed into Escherichia coli DH5. The resultant transformants was screened by restriction **enzyme digestion** of individual DNA preparations. The desired clone, with the 3' end of the mu cDNA cloned into Bluescript was called pBmu3'. A complete PstI and BamHI digestion of pABmu-11 generated a PstI-BamHI fragment of approximately 870 base pairs , that was purified by elution from a low-melt agarose gel. This fragment, called mu5', contained the 5' end of the mucDNA, with the exception of the leader sequence. Another fragment, called mu3', was prepared from pBmu3', by digestion with BamHI and EcoRI, and elution from a low-melt agarose gel. This fragment of approximately 1 kb contained the 3'p sequence derived from pABmu-11, with an EcoRI site at the 3' end of the Bluescript polylinker sequence. Fragments mu5' and mu3' were ligated with EcoRI-digested pMT3STVA, and two synthetic oligodeoxyribonucleotides, to reconstruct the leader sequence. The sequences of exemplary synthetic oligodeoxyribonucleotides were S1 and S2. The ligation products were transformed into E. coli DH5, and transformants were screened by colony hybridization to one of these two oligodeoxyribonucleotides labeled with 32P, using standard procedures. Positive colonies were characterized further with restriction **enzyme digestion** analysis of DNA preparations.

Digestions with SalI and enzymes that cut in the cDNA, such as BglII and BamHI was used to orientate the insert cloned into the vector, for a unique SalI site positioned 3' to the EcoRI site in pMT3SVA. pMT3Amuf was produced. The lambda chain was introduced into an expression vector to produce pAdlambda. The mouse immunoglobulin lambda , light chain used was derived from pABlambda-15. Initially the PstI fragment from this plasmid

bearing the lambda1 cDNA was cloned into the PstI site of pSP65N, to give plambda1-3. The vector, pSP65N, was derived from the pSP65 by digestion with HindIII, enzymatic filling-in of the HindIII cohesive ends, and ligation with NotI linkers. The ligation products were digested with NotI, and religated to generate pSP65N. plambda1-3 was digested with FokI and SalI, and the two new bands of approximately 307 bp (I) and 550 bp (II) were excised from a low-melt agarose gel, and purified. The expression vector used was derived from pMT2DGR. This plasmid was digested with SalI and XhoI, and the desired vector fragment was distinguished from the other fragment bearing factor VIII-related sequences on a low-melt agarose gel, and the vector fragment was excised and purified. To create pADlambda1, the pMT2DGR-derived vector fragment was ligated with fragments (I) and (II), and two synthetic oligodeoxyribonucleotides of S3 and S4. These synthetic sequences annealed to each other, and to the FokI cohesive end of the 5' end of (I). Their nucleotide sequence reconstructed the 5' end of the coding region and created a small, 5' untranslated region. The ligation products were transformed into E. coli DH5, and the desired recombinants were identified by restriction **enzyme digestion** of small scale DNA preparations from individual transformants. pMT3Amuf and pADlambda1 were separately electroporated into separate pools of **CHO** DUKX cells (which were dhfr-). Pools of transfected clones were made and selected in increasing concentrations of 2'-deoxycoformycin (dCF) or methotrexate (MTX), respectively. Two pools selected at 3 microM dCF (mu) or 50 nM MTX (lambda) were fused by conventional methods in polyethylene glycol, and ADA+ DHFR+ ells were selected up to 3 micro Molar dCF (mu) and 50 nM MTX. The cells were then further selected up to 3 micro Molar dCF and 200 micro Molar MTX and 10 micro Molar dCF and 50 nM MTX. It was found that only the increased concentration of dCF led to an increase in the amount of functional antibody (Ab) as determined by a hapten-binding enzyme linked immunosorbant assay (ELISA). This correlated with an increase in the amount of heavy chain produced, and therefore it was concluded that the amount of heavy chain was limiting the amount of functional antibody produced. The 10 micro Molar dCF and 50 nM MTX pool was then further selected at up to 40 micro Molar dCF and 50 nM MTX. At this stage, clones were obtained by plating the cells at low density, and after an appropriate period of growth, macroscopic colonies were cloned out using cloning cylinders. The levels of mu, lambda and NP-binding monoclonal Ab produced at different levels of selection were measured by ELISA. 5'-aattcgtaatgggatggagctgtatcatgctcttcttggcagcaacagctacaggtgtccact ccaggtccaactgca-3' (S1) 5'-gttgacctgggagtggacacctgtagctgttgctgccaagaaga gcatgatacagctccattcccattag-3' (S2) 5'-tcgacgccatggcctggatt-3' (S3) 5'-gtgaaatccaggccatggccg-3' (S4)(5 pages)

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     (FILE 'HOME' ENTERED AT 11:05:41 ON 19 JUN 2006)

     FILE 'CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ESBIOBASE' ENTERED AT
     11:06:24 ON 19 JUN 2006
L1             0 S (ANTIBODY FRAGMENT) AND CHO AND PEPSIN
L2           681 S (ANTIBODY FRAGMENT) AND CHO
L3          1487 S (ASPARTYL PROTEASE) OR (ASPARTYL ENZYME)
L4             2 S L2 AND L3
L5             1 DUPLICATE REMOVE L4 (1 DUPLICATE REMOVED)
L6          8803 S (ENZYME DIGESTION) OR (PROTEASE DIGESTION)
L7             1 S L2 AND L6

=> s (endogenous enzyme) and l2
L8             2 (ENDOGENOUS ENZYME) AND L2

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DUPLICATE PREFERENCE IS 'CAPLUS, BIOTECHDS'
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=> d l9 bib abs 1

L9   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2006 ACS on STN  DUPLICATE 1
AN   2004:354964  CAPLUS
DN   140:373903
TI   Method for cleaving antibodies and generating antibody
     fragments
IN   Zapata, Gerardo A.
PA   Abgenix, Inc., USA
SO   PCT Int. Appl., 21 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
PI   WO 2004035608         A2    20040429    WO 2003-US33121         20031017
     WO 2004035608         A3    20040812
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             OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
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             BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
     AU 2003286493         A1    20040504    AU 2003-286493          20031017
     US 2004138428         A1    20040715    US 2003-688198          20031017
PRAI US 2002-419908P       P     20021018
     WO 2003-US33121       W     20031017
AB   The disclosed invention is related to a method for producing
     antibody fragments. In particular, the invention
     involves a method for the production of F(ab')2 fragments. The method
     comprises concentration of cell culture media and activation of
     endogenous enzymes present in the cell culture media by
     adjusting the temperature and pH. Cell cultures expressing Igs can be selected
     from hybridoma or CHO cell cultures. For the production of the
     antibody cells are grown in stirred tank bioreactors with appropriate
     media. Cell culture supernatant is harvested and filtered prior to being
     subjected to pH treatment and activation of enzymic cleavage. Enzymes
     responsible for the enzymic activity in the activated cell culture media
     are aspartyl and cysteinyl proteases. For purification of the F(ab')2
     fragments generated in the digestion reaction, the cell culture media are
     further subjected to several chromatog. steps including a Q Sepharose FF
     column, a protein A column, and a hydrophobic interaction chromatog. (HIC)
     column.


=> s ((antibody fragment) and (production or producing))
L10        3041 ((ANTIBODY FRAGMENT) AND (PRODUCTION OR PRODUCING))

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L1             0 S (ANTIBODY FRAGMENT) AND CHO AND PEPSIN
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L2          681 S (ANTIBODY FRAGMENT) AND CHO
L3         1487 S (ASPARTYL PROTEASE) OR (ASPARTYL ENZYME)
L4            2 S L2 AND L3
L5            1 DUPLICATE REMOVE L4 (1 DUPLICATE REMOVED)
L6         8803 S (ENZYME DIGESTION) OR (PROTEASE DIGESTION)
L7            1 S L2 AND L6
L8            2 S (ENDOGENOUS ENZYME) AND L2
L9            1 DUPLICATE REMOVE L8 (1 DUPLICATE REMOVED)
L10        3041 S ((ANTIBODY FRAGMENT) AND (PRODUCTION OR PRODUCING))

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L11          12 L6 AND L10

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KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
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=> d l12 bib abs 1-9

L12   ANSWER 1 OF 9  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN    2005-09542  BIOTECHDS
TI    An engineered chaperonin caging a guest protein: Structural insights and
      potential as a protein expression tool;
         chaperonin-caged protein expression system for recombinant protein
         production and protease-catalyzed proteolysis prevention
AU    FURUTANI M; HATA JI; SHOMURA Y; ITAMI K; YOSHIDA T; IZUMOTO Y; TOGI A;
      IDENO A; YASUNAGA T; MIKI K; MARUYAMA T
CS    Sekisui Chem Co Ltd; Kyoto Univ; Kyushu Inst Technol; JAMSTEC; RIKEN;
      Marine Biotechnol Inst
LO    Furutani M, Sekisui Chem Co Ltd, Inst Res and Dev, Hyakuyama 2-1,
      Mishima, Osaka 6188589, Japan
SO    PROTEIN SCIENCE; (2005) 14, 2, 341-350    ISSN:  0961-8368
DT    Journal
LA    English
AN    2005-09542  BIOTECHDS
AB    AUTHOR ABSTRACT - The structure of a chaperonin caging a substrate
      protein is not quite clear. We made engineered group H chaperonins fused
      with a guest protein and analyzed their structural and functional
      features. Themococcus sp. KS-1 chaperonin alpha-subunit (TCP) which forms
      an eightfold symmetric double-ring structure was used. Expression
      plasmids were constructed which carried two or four TCP genes ligated
      head to tail in phase and a target protein gene at the 3' end of the
      linked TCP genes. Electron microscopy showed that the expressed gene
      products with the molecular sizes of Crsimilar to120 kDa (di-TCP) and
      similar to230 kDa (tetra-TCP) formed double-ring complexes similar to
      those of wild-type TCP. The tetra-TCP retained ATPase activity and its
      thermostability was significantly higher than that of the wild type. A
      260-kDa fusion protein of tetra-TCP and green fluorescent protein (GFP,
      27 kDa) was able to form the double-ring complexes with green
      fluorescence. Image analyses indicated that the GFP moiety of
      tetra-TCP/GFP fusion protein was accommodated in the central cavity, and
      tetra-TCP/GFP formed the closed-form similar to that crystallographically
      resolved in group II chaperonins. Furthermore, it was suggested that
      caging GFP expanded the cavity around the bottom. Using this tetra-TCP
      fusion strategy, two virus structural proteins (21-25 kDa) toxic to host
      cells or two antibody fragments (25-36 kDa) prone to
      aggregate were well expressed in the soluble, fraction of Escherichia
      coli. These fusion products also assembled to double-ring complexes,
      SU22esting encapsulation of the guest proteins. The antibody
      fragments liberated by site-specific protease
      digestion exhibited ligand-binding activities. (10 pages)
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L12 ANSWER 2 OF 9 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-01750 BIOTECHDS
TI Novel recombinant protein disulfide isomer, useful for treating congestive heart failure, endotoxic shock, Crohn's disease, and autoimmune diseases, such as thyroiditis and rheumatoid and osteo-arthritis;
antibody **production** against protein for use in disease therapy and diagnosis
AU MOZIER N M; DUFIELD R L; MO J; BILD G S
PA PHARMACIA CORP
PI WO 2003080674 2 Oct 2003
AI WO 2003-US8608 20 Mar 2003
PRAI US 2002-366350 20 Mar 2002; US 2002-366350 20 Mar 2002
DT Patent
LA English
OS WPI: 2003-865093 [80]
AN 2004-01750 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A recombinant protein disulfide isomer, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a therapeutic or diagnostic composition (I) comprising a recombinant protein and a disulfide isomer of the protein in combination with an excipient, diluent or carrier; (2) analyzing (M1) the characterization and quantitation of **antibody fragment** disulfide isomers comprises: (a) pre-treating the **antibody fragment** with an organic solvent; (b) digesting the pre-treated **antibody fragment** with proteases in the presence of the organic solvent; and (c) a unit resolving the protease digest fragments; and (3) analyzing (M2) the characterization and quantitation of **antibody fragment** degradation products and **antibody fragment** impurities in recombinant proteins chosen from methionyl oxidations, truncations, deamidation of asparagines, misincorporations, extensions or others involves pre-treating the protein with an organic solvent, digesting the pre-treated protein with proteases in the presence of the organic solvent and a unit resolving the protease digest fragments.

BIOTECHNOLOGY - Preferred Disulfide Isomer: The recombinant protein is an antibody preferably Fab and has specificity for tumor necrosis factor-alpha (TNFalpha). Fab comprises a modified Fab fragment where the modification is the addition to the C-terminal end of its heavy chain one or more amino acids such as cysteine to allow the attachment of an effector or reporter molecule. The effector is a polymer such as branched polyethylene glycol (PEG). TNFalpha antibody is CDP870 where the disulfide linkage is between Cys 214 of the light chain and Cys 227 of the heavy chain of CDP870. Preferred Composition: The disulfide isomer is 1%-60%, 10%-20%, less than 20%, less than 15% or preferably 13% of the total antibody concentration. Preferred Method: In (M1) and (M2) the organic solvent is acetonitrile. In (M1), the acetonitrile concentration is 40-80%, preferably 67% in the pre-treatment of **antibody fragment** and 20-50%, preferably 20% in the **protease digestion** of pre-treated **antibody fragment**.
The protease is Lys-C or trypsin. The unit resolving the protease digest fragments is reversed phase high performance liquid chromatography (HPLC). The **antibody fragment** is an Fab, modified Fab, Fab', F(ab')2 or Fv fragment, a light chain or heavy chain monomer or dimer or a single chain antibody, or is preferably a Fab fragment which is CDP870. In (M2), the acetonitrile concentration is 50% in the pre-treatment of the protein and 20% in the **protease digestion** of the pre-treated protein.

ACTIVITY - Antibacterial; Immunosuppressive; Cardiovascular-Gen.; Immunomodulator; Respiratory-Gen.; Antiinflammatory; Anti-HIV; Antipsoriatic; Antitubercular; Tuberculostatic; Anticoagulant; Hemostatic; Vulnerary; Antithyroid; Antirheumatic; Antiarthritic; Osteopathic.

MECHANISM OF ACTION - TNF alpha reducer. No supporting data is given.

USE - The recombinant protein is useful as a therapeutic and diagnostic agent (claimed). The recombinant protein which is an antibody is useful in the treatment of sepsis, congestive heart failure, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, AIDS, allergies, psoriasis, tuberculosis (TB), inflammatory bone disorders, blood coagulation disorders, burns, rejection episodes following organ or tissue transplant, Crohn's disease and autoimmune diseases, such as thyroiditis and rheumatoid and osteo-arthritis. The antibody is also useful to reduce side effects associated with TNF alpha generation during neoplastic therapy, to eliminate or reduce shock-related symptoms associated with the treatment or prevention of graft rejection by use of an anti-lymphocyte antibody, or for treating multi-organ failure.

ADMINISTRATION - (I) is administered by oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricluar, transdermal, transcutaneous, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. The effective dosage is 0.01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 20 mg/kg, more preferably 15 mg/kg.

EXAMPLE - Tumor necrosis factor alpha (TNFalpha) affinity column was prepared by coupling human recombinant TNF alpha to a bio-support medium. TNF alpha was dissolved in coupling buffer, 0.6 M sodium citrate and 50 mM CHES, at pH 9.0. To make a 1 ml column, 0.126 g (dry weight) of bio-support was incubated with 750 microl of TNFalpha in coupling buffer. The reaction was incubated for 72 hours at room temperature with mixing. The beads were centrifuged. The supernatant was decanted and the resin was resuspended in 10 ml of quench buffer and incubated for 2.5 hours at room temperature in a 15 ml screw top conical with gentle inversion. Quench buffer was 3 M ethanolamine, pH 9.0. After quenching the conical was centrifuged for 10 minutes, and the supernatant was decanted and the resin was resuspended in 10 ml of phosphate buffered saline (PBS) with pH 7.4 for 20 minutes at room temperature with gentle inversion. The washed beads were centrifuged for 10 minutes, the supernatant was decanted, and the beads were re-suspended in 10 ml of 1 M sodium chloride and incubated for 20 minutes at room temperature with gentle mixing. The beads were centrifuged again, the supernatant was decanted, and the beads were re-suspended in 10 ml PBS, pH 7.4. This was mixed for 20 minutes, and the above mentioned step was repeated. The beads were then resuspended in 4 ml of PBS, pH 7.4 and poured into a HR 5/5 column. The resulting 1 ml column was equilibrated in 50 column volumes (cv) of buffer A. Buffer A was 10 mM -4 -(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), 150 mM sodium chloride, pH 7.4. The column was equilibrated and washed in 10 mM HEPES, 150 mM sodium chloride, pH 7.4. The column was loaded with CDP870 (36426803, 200 mg/ml stock) that was diluted to 2 mg/ml with Buffer A and injected 0.5 ml/run. The column was eluted isocratically with 100 mM glycine, pH 3.4. The column was cleaned with 10 mM HEPES, 2 M sodium chloride, pH 7.4. After loading, the flow-through (unbound) and also the eluted (bound) fractions were collected. The two fractions and the column feed material were analyzed by LysC digestion followed by mass spectrometry.(40 pages)

L12 ANSWER 3 OF 9 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-02085 BIOTECHDS
TI Novel nucleic acid sequence encoding antigen-binding site of the heavy or light chain of an antibody useful for expressing the antibody suitable in the field of tumor diagnostics and therapeutics;
vector-mediated recombinant antibody gene transfer and expression in host cell for use in humanized antibody, single chain antibody, radionuclide, cholera toxin and ricin preparation
AU BOLHUIS R L; WOEHL T; BOETTGER V
PA WILEX AG
PI WO 2002063010 15 Aug 2002
AI WO 2002-EP1283 7 Feb 2002

PRAI US 2001-327008 5 Oct 2001; US 2001-266853 7 Feb 2001
DT Patent
LA English
OS WPI: 2002-627562 [67]
AN 2003-02085 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Nucleic acid (I) encoding the antigen-binding site of the heavy or light chain of an antibody comprising a nucleotide sequence encoding the CDR3 region, is new.

DETAILED DESCRIPTION - Nucleic acid (I) encoding the antigen-binding site of the heavy or light chain of an antibody comprising a nucleotide sequence encoding the CDR3 region, is new. (I) encodes the antigen-binding site of the heavy chain of an antibody comprising a nucleotide sequence encoding the CDR3 region: His-Arg-Ser-Gly-Tyr-Phe-Ser-Met-Asp-Tyr (designated H3), or the light chain of an antibody comprising a nucleotide sequence encoding the CDR3 region: Gln-Gln-Tyr-Ser-Asn-Tyr-Pro-Trp-Thr (designated L3). An INDEPENDENT CLAIM is included for a recombinant vector comprising at least a copy of (I);

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) comprises a nucleotide sequence encoding the CDR2 region: Ala-Ile-Asn-Ser-Asp-Gly-Gly-Ile-Thr-Tyr-Tyr-Leu-Val-Thr-Val-Lys-Gly (designated H2), or Ser-Ala-Ser-Asn-Arg-Tyr-Thr (designated L2), or CDR1 region: Asn-Tyr-Tyr-Me-Ser (designated H1), or Lys-Ala-Ser-Gln-Asn-Val-Val-Ser-Ala-Val-Ala (designated L1).

USE - (I) is useful for the recombinant **production** of a polypeptide having an antigen-binding site, by introducing (I) into a mammalian cell, culturing the cell in a medium where an expression of the nucleic acid takes place, and obtaining the expressed product from the medium and/or the cell. Before introducing (I) into the cell, the nucleic acid is modified so that the modification does not alter the amino acid sequence of the antigen-binding site of the polypeptide to be expressed. The expressed product, preferably antibodies and **antibody fragments** are useful for preparing a diagnostic or therapeutic agent and are coupled to a diagnostic marker or cytotoxic agent. (All claimed). (I) is useful for expressing antibodies, e.g. chimerized antibodies, humanized antibodies, heterobispecific antibodies, and single chain antibodies, or **antibody fragments**. The expressed product can be coupled to a diagnostic marker for use in in vitro diagnostic methods, radioimaging procedures, or to a cytotoxic agent, e.g. a radionuclide, or a toxin such as cholera toxin or ricin.

EXAMPLE - G250 tumor-associated antigen-specific immunoglobulin variable heavy (VH) and light (VL) chain domains were isolated, cloned and sequenced from the G250 monoclonal antibody **producing** hybridoma. The variable region genes for the heavy and light chains, which determine the binding specificity of the antibody, were cloned from the G250 murine hybridoma using standard cloning techniques. The strategy for cloning the variable regions for the heavy and light chain genes from the G250 hybridoma was achieved by polymerase chain reaction (PCR) amplification of cDNA obtained from the G250 monoclonal antibody **producing** hybridoma cells. Obtaining the G250 VH and VL chain sequences from the G250 monoclonal antibody **producing** hybridoma was achieved by PCR amplification of cDNA obtained from the respective clone. To obtain cDNA, total RNA was isolated from the G250 **producing** hybridoma cells, converted into cDNA, and amplified. For PCR two primers complementary to the 5'-end and the 3'-end of the sequence were used as the initiation point of DNA synthesis. Because the sequence of the 5'-ends of the VH and VL chain from the G250 monoclonal antibody **producing** hybridoma cells were unknown, the PCR method, referred to as RACE (rapid amplification of cDNA ends) was used to amplify the VH and VL chain. This was achieved by employing anchor (5'-GCATGCGCGCGGCCGCGGAGGCC-3') and anchor-poly-C (5'-GCATGCGCGCGGCCGCGGAGGCC(C)(12)-3') primers and the constant VH (CTCTAAGCTTGGCTCAAACACAGCGACCTCGGATACAGTTGGTGCAGC-3') and VL-primers (5'-CTCTTCTAGAGAGTCTCTCAGCTGGTAGGATACAGTTGGTGCAGC-3'). The VH and VL fragments were purified and ligated into pGEM11. A ligation mixture was

introduced into bacteria, which were selected and expanded. DNA was isolated from the selected bacterial colonies and analyzed by restriction **enzyme digestion** to confirm the presence of the amplified VH and VL fragments. Three positive colonies were subjected to DNA sequencing. The sequences of these three individual clones were compared and found to be identical.(18 pages)

L12 ANSWER 4 OF 9 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2002-18803 BIOTECHDS
TI New tumor markers comprising SC5 and the p140 allelic form, useful as universal markers for cutaneous T cell lymphomas (CTCL), particularly useful for diagnosing CTCL, as well as psoriasis, eczema, dermatitis, or non-CTCL T lymphomas;
vector-mediated recombinant protein gene transfer and expression in host cell for use in disease diagnosis and gene therapy
AU BENSUSSAN A; BOUMSELLE L; BAGOT M; MORETTA A
PA INSERM INST NAT SANTE and RECH MEDICALE
PI WO 2002050122 27 Jun 2002
AI WO 2000-EP15417 18 Dec 2000
PRAI EP 2000-403580 18 Dec 2000
DT Patent
LA English
OS WPI: 2002-508789 [54]
AN 2002-18803 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Molecules comprising SC5 and an allelic form of p140, which are markers for cutaneous T cell lymphomas (CTCL), are new, where SC5 is the antigen of a monoclonal antibody (mAb) produced a hybridoma (I-2575) deposited at C.N.C.M, and the allelic form of p140 has a DNA sequence of 1395 base pairs (I) encoding a 434 amino acid (II) sequence, both given in the specification.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a hybridoma deposited at C.N.C.M. as I-2575; (2) mAb produced by the hybridoma; (3) isolated cDNA obtained by using the mAb; (4) isolated mRNA obtainable by selecting an mRNA population of total blood cells and peripheral blood lymphocytes (PBL), where the mRNA is complementary to the cDNA; (5) isolated genomic DNA encoding the mRNA; (6) an engineered cell in which the cDNA, mRNA or DNA has been transfected; (7) an isolated protein or polypeptide obtained by using the mAb, or encoded by the cDNA, mRNA or DNA; (8) a bank of polypeptide compounds obtainable by cleavage of (7) with an enzyme consisting of V8 protease or alpha chymotrypsin; (9) a fragment of the mAb comprising heavy and light chains, VH, VL, Fab, F(ab')2, CD1, CDR2 or CDR3; (10) an isolated compound comprising the fragment; (11) an isolated protein comprising (II), and an isolated DNA comprising (I) or encoding the protein; (12) a polypeptidic vector having an element comprising the mAb, the Fab or F(ab')2 fragments or the isolated compound; (13) a medical kit comprising the polypeptidic vector and an anti-mitotic pro-drug (e.g. phenol mustard pro-drug); (14) a medicament comprising as an active principle the mAb, the Fab or F(ab')2 fragments, or humanized antibodies; (15) evaluating the percentage of malignant CD4+ CTCL cells present within a certain body compartment of a patient; (16) methods for CTCL diagnosis; (17) a kit for assessing the development level of a CTCL or for CTCL diagnosis comprising any of the following: (a) the mAb, Fab or F(ab')2 fragments, or humanized antibodies; (b) the bank of polypeptide compounds; (c) the cDNA or mRNA; (d) the p140 binding compounds or p140 DNA; or (e) the DNA comprising 1935 bp and encoding 434 amino acids (III), the sequences of which are given in the specification; and (18) identifying a compound useful in the palliation, prevention, relief or therapy of malignant CTCL cells, where the compound is capable of binding to the protein, to the polypeptide compound of (8), or to the p140 molecule comprising (II) or (III).

BIOTECHNOLOGY - Preferred Compounds: The polypeptidic vector comprises a p140 binding domain. It further comprises tumoral toxins or

radioelements. It also comprises an enzyme capable of transforming an anti-mitotic pro-drug into an active drug form, e.g. carboxypeptidase. The mAb is obtainable by: (a) immunizing an animal against the SC5 protein or polypeptide; (b) **producing** hybridomas from the spleen cells of this animal, and cultivating them to produce monoclonal antibodies in their supernatants; (c) evaluating the supernatants from the presence of an antibody, which is capable of binding to the protein or polypeptide compound that has been used as an immunogen in step (a), and which has a property consisting of: (1) binding resting non-tumoral T cells mainly in their cytoplasmic compartment, and binding malignant CD4+ CTCL cells mainly at their cell surface; (2) modulating the CD3 activation pathway of T cells; (3) modulating interleukin (IL)-2 **production** from T cells; (4) modulating CD3-induced proliferation of T cells; (5) modulating the CD3-induced in vitro proliferation of CTCL cells; (6) modulating the proliferation of CTCL cells in a non-human animal; or (7) competing with the mAb for binding to the isolated protein or against the polypeptide compound; (d) selecting and cloning hybridomas **producing** the desired antibody; and (e) recovering the antibody from the supernatant. The compound or **antibody fragment** is a humanized antibody. It further comprises an anti-CD4 fragment consisting of heavy chains, light chains, VH, VL, Fab, F(ab')2, CD1, CDR2 or CDR3 fragments of anti-CD4 antibodies. Preferred Method: In method (15), evaluating the percentage of malignant CD4+ CTCL cells present in a patient's body comprises measuring the percentage of CD4+ cells expressing the SC5 protein or polypeptide, or p140 molecules in a biological sample collected from the patient. The percentage of malignant CD4+ CTCL cells actually present in the body is considered as falling within a +/- 10 % range around the measured percentage. In method (16), the CTCL diagnosis is characterized in that the percentage of T cells expressing the SC5 protein or polypeptide, or p140 molecules is measured in a biological sample collected from the patient, and is then compared to the average percentage observed in non-CTCL humans. A CTCL-positive diagnosis is decided when the measured percentage is significantly higher than the average percentage. The method may also involve measuring the percentage of CD4+ cells expressing the SC5 protein or polypeptide in a biological sample collected from a patient. A CD4+ CTCL positive diagnosis is decided when the measured percentage is higher than the average percentage usually observed in non-CTCL humans. The presence of CD4+ cells expressing p140 is also searched in a biological sample comprising potentially CTCL cells. A CTCL-positive diagnosis is concluded when the presence is significantly detected. The percentage measurement makes use of the mAb, the Fab or F(ab')2 fragments, the humanized antibodies comprising the Fab or F(ab')2 fragments, the bank of polypeptides, the SC5 cDNA or mRNA, the SC5, the p140 binding compounds, the p140 DNA or p140 polypeptides. Preparation: The isolated protein described in (7), which is the SC5, is obtainable by: (a) collecting cells from total blood cells and PBL, and stimulating them with phytohaemagglutinin (PHA) at 1 microgram/ml; (b) lysing the cells by incubation in a lysis buffer containing Triton X-100 (RTM) at 1 %; and (c) recovering from the lysate the compound onto which the mAb binds under conditions enabling this mAb to perform reactions of the antigen-antibody type. The protein is obtained by recovering the isolated protein under non-reducing conditions, e.g. via chromatography by affinity with the mAb. Specifically, the polypeptide is obtainable by: (a) collecting PBL cells or total blood cells; (b) incubating the collected cells with a CD3 activator, e.g. PHA at 1 microgram/ml; (c) labeling the cells with a polypeptide-specific label, e.g. biotin; (d) lysing the cells in a lysis buffer comprising Triton X-100 (RTM) at 1 %; (e) submitting the lysate to an **enzyme digestion** with an enzyme selected from V8 protease or alpha chymotrypsin; (f) submitting the lysate to an immuno-precipitation reaction with the mAb; and (g) recovering from the digested immuno-precipitate any compound that bears the label. The isolated cDNA is obtainable by: (a) collecting cells from total blood cells and PBL; (b) incubating the collected cell population

with a CD3 activator, e.g. PHA at 1 microgram/ml; (c) extracting and purifying the whole mRNA population from the cells; (d) synthesizing every complementary cDNA; (e) operably cloning each cDNA so that expression of this cDNA in this clone is possible under appropriate clone culture conditions, and cultivating every clone accordingly; (f) selecting those clones that express a compound onto which the mAb binds, and/or of which lysate would give an immuno-precipitation reaction with this mAb; (g) optionally, amplifying those cloned that have been selected; and (h) recovering the inserted cDNA from the selected clones.

ACTIVITY - Cytostatic. No suitable biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - The SC5 and the p140 allelic form are useful as tumor markers, particularly as universal markers for CTCL. These are particularly useful for diagnosing CTCL, as well as skin immune mediated disease (e.g. psoriasis, eczema or dermatitis) and non-CTCL T lymphomas. The polypeptidic vector is useful for treating CTCL (claimed). The p140 binding compound is useful for the manufacture of an anti-CTCL medicament (claimed).

EXAMPLE - Experimental protocols are described but no results are given. (91 pages)

L12 ANSWER 5 OF 9 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-09763 BIOTECHDS
TI Optimizing monoclonal antibody expression level, comprises **producing** a host cell having DNA sequences encoding antibody heavy and light chains, with an amplifiable marker, culturing the host, and amplifying sequences;
hybridoma cell culture and plasmid expression in Escherichia coli
AU WOOD C R; KAUFMAN R J
PA WYETH
PI US 6475787 5 Nov 2002
AI US 1991-765281 25 Sep 1991
PRAI US 1991-765281 25 Sep 1991; US 1989-386489 28 Jul 1989
DT Patent
LA English
OS WPI: 2003-208838 [20]
AN 2003-09763 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Optimizing (M) a monoclonal antibody expression level, by: (a) **producing** a eukaryotic host cell (HC) capable of expressing 2 DNA sequences (DSs) encoding antibody heavy and light chains, where the DSs are associated with heterologous selectable amplifiable marker genes; (b) culturing HC; (c) measuring the relative amounts of DSs expressed; and (d) differentially amplifying DSs with appropriate selective agents.

DETAILED DESCRIPTION - Optimizing (M) the expression level of a monoclonal antibody, comprises: (a) **producing** a eukaryotic host cell containing and capable of expressing two DNA sequences encoding an antibody heavy and light chain, respectively, where the two DNA sequences are associated with two heterologous selectable amplifiable marker genes, respectively; (b) culturing the host cell in a suitable culture medium; (c) measuring the relative amounts of the two DNA sequences expressed; and (d) differentially amplifying the amounts of the 2 DNA sequences with appropriate selective agents to allow maximized **production** of the antibody. An INDEPENDENT CLAIM is also included for a host cell (I) produced by (M).

USE - (M) is useful for optimizing the expression level of a monoclonal antibody (claimed). (M) is useful for **producing** monoclonal antibodies. (M) is useful for the **production** of cloned antibodies, genetically engineered antibodies such as complementarity determining region (CDR)-swapped antibodies, genetically engineered **antibody fragments** or derivatives, and hetero-dimeric and complete antibodies.

ADVANTAGE - (M) is an improved method for **production** of monoclonal antibodies.

EXAMPLE - Heavy and light chain cDNAs were cloned from the B1-8 hybridoma cell line (a fusion of a mouse splenocyte and a murine myeloma cell line which produces an immunoglobulin (Ig)M antibody directed to the hapten, 4-hydroxy-3-nitrophenyl acetate). The mu chain DNA and the lambda A chain DNA was each isolated as restriction fragments. The mu chain cDNA was cloned into plasmid pMT3SVA as follows to produce pMT3Amu. The heavy chain expression plasmid was constructed with the p heavy chain cDNA of pABmu-11. The mu cDNA was isolated and prepared for cloning into the EcoRI site of the expression vector pMT35SVA as follows. pABmu-11 was digested to completion with BglII, and then a partial PstI digestion was performed. One resulting BglII-PstI fragment of approximately 1 kb containing the complete 3' end of the cDNA was purified from a low-melt agarose gel. This was then ligated into BamHI and PstI digested Bluescript plasmid and transformed into Escherichia coli DH5. The resultant transformants was screened by restriction **enzyme digestion** of individual DNA preparations. The desired clone, with the 3' end of the mu cDNA cloned into Bluescript was called pBmu3'. A complete PstI and BamHI digestion of pABmu-11 generated a PstI-BamHI fragment of approximately 870 base pairs , that was purified by elution from a low-melt agarose gel. This fragment, called mu5', contained the 5' end of the mucDNA, with the exception of the leader sequence. Another fragment, called mu3', was prepared from pBmu3', by digestion with BamHI and EcoRI, and elution from a low-melt agarose gel. This fragment of approximately 1 kb contained the 3'p sequence derived from pABmu-11, with an EcoRI site at the 3' end of the Bluescript polylinker sequence. Fragments mu5' and mu3' were ligated with EcoRI-digested pMT3STVA, and two synthetic oligodeoxyribonucleotides, to reconstruct the leader sequence. The sequences of exemplary synthetic oligodeoxyribonucleotides were S1 and S2. The ligation products were transformed into E. coli DH5, and transformants were screened by colony hybridization to one of these two oligodeoxyribonucleotides labeled with 32P, using standard procedures. Positive colonies were characterized further with restriction **enzyme digestion** analysis of DNA preparations.
Digestions with SalI and enzymes that cut in the cDNA, such as BglII and BamHI was used to orientate the insert cloned into the vector, for a unique SalI site positioned 3' to the EcoRI site in pMT3SVA. pMT3Amuf was produced. The lambda chain was introduced into an expression vector to produce pAdlambda. The mouse immunoglobulin lambda , light chain used was derived from pABlambda-15. Initially the PstI fragment from this plasmid bearing the lambda1 cDNA was cloned into the PstI site of pSP65N, to give plambda1-3. The vector, pSP65N, was derived from the pSP65 by digestion with HindIII, enzymatic filling-in of the HindIII cohesive ends, and ligation with NotI linkers. The ligation products were digested with NotI, and religated to generate pSP65N. plambda1-3 was digested with FokI and SalI, and the two new bands of approximately 307 bp (I) and 550 bp (II) were excised from a low-melt agarose gel, and purified. The expression vector used was derived from pMT2DGR. This plasmid was digested with SalI and XhoI, and the desired vector fragment was distinguished from the other fragment bearing factor VIII-related sequences on a low-melt agarose gel, and the vector fragment was excised and purified. To create pADlambda1, the pMT2DGR-derived vector fragment was ligated with fragments (I) and (II), and two synthetic oligodeoxyribonucleotides of S3 and S4. These synthetic sequences annealed to each other, and to the FokI cohesive end of the 5' end of (I). Their nucleotide sequence reconstructed the 5' end of the coding region and created a small, 5' untranslated region. The ligation products were transformed into E. coli DH5, and the desired recombinants were identified by restriction **enzyme digestion** of small scale DNA preparations from individual transformants. pMT3Amuf and pADlambda1 were separately electroporated into separate pools of CHO DUKX cells (which were dhfr-). Pools of transfected clones were made and selected in increasing concentrations of 2'-deoxycoformycin (dCF) or methotrexate (MTX), respectively. Two pools selected at 3 microM dCF (mu) or 50 nM MTX (lambda) were fused by conventional methods in polyethylene

glycol, and ADA+ DHFR+ ells were selected up to 3 micro Molar dCF (mu) and 50 nM MTX. The cells were then further selected up to 3 micro Molar dCF and 200 micro Molar MTX and 10 micro Molar dCF and 50 nM MTX. It was found that only the increased concentration of dCF led to an increase in the amount of functional antibody (Ab) as determined by a hapten-binding enzyme linked immunosorbant assay (ELISA). This correlated with an increase in the amount of heavy chain produced, and therefore it was concluded that the amount of heavy chain was limiting the amount of functional antibody produced. The 10 micro Molar dCF and 50 nM MTX pool was then further selected at up to 40 micro Molar dCF and 50 nM MTX. At this stage, clones were obtained by plating the cells at low density, and after an appropriate period of growth, macroscopic colonies were cloned out using cloning cylinders. The levels of mu, lambda and NP-binding monoclonal Ab produced at different levels of selection were measured by ELISA. 5'-aattcgtaatgggatggagctgtatcatgctcttcttggcagcaacagctacaggtgtccact ccaggtccaactgca-3' (S1) 5'-gttgacctgggagtggacacctgtagctgttgctgccaagaaga gcatgatacagctccattcccattag-3' (S2) 5'-tcgacgccatggcctggatt-3' (S3) 5'-gtgaaatccaggccatggccg-3' (S4)(5 pages)

L12 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
AN 1999:319669 CAPLUS
DN 131:169014
TI Selection of rabbit single-chain Fv fragments against the herbicide atrazine using a new phage display system
AU Li, Yi; Cockburn, William; Kilpatrick, John; Whitelam, Garry C.
CS Department of Biology, University of Leicester, Leicester, LE1 7RH, UK
SO Food and Agricultural Immunology (1999), 11(1), 5-17
CODEN: FAIMEZ; ISSN: 0954-0105
PB Carfax Publishing Ltd.
DT Journal
LA English
AB A convenient new bacteriophage display vector, pSD3, has been constructed and used to generate rabbit monoclonal anti-pesticide **antibody fragments**. Following amplification of Ig light chain, and heavy chain variable region gene libraries, restriction enzymes Sfi I and PflM I are used to assemble scFv libraries in pSD3. This allows the number of stages involving the polymerase chain reaction and restriction **enzyme digestion** to be minimized to optimize maintenance of the original diversity of the variable region genes in the libraries. The vector also incorporates an amber codon, a 6xHis tag and a c-myc epitope to facilitate soluble single-chain Fv **prodn**. detection and purification Using the pSD3 system two anti-atrazine single-chain Fvs were isolated from a library derived from the spleen cells of a rabbit immunized with bovine serum albumin-atrazine conjugate. Characterization of single-chain Fvs by competition and equilibrium ELISA indicated good specificity and affinity to atrazine.
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 9 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 1997-02389 BIOTECHDS
TI Construction of a vector which displays the fragment of an antibody on the surface of a filamentous phage particle;
Escherichia coli transformation with phagemid pAIg 316 for Z22 anti-Z-DNA antibody scFv fragment and phage M13 fusion protein expression; phage display
AU Queiroz Maranhao A
CS Univ.Brasilia
LO Laboratorio de Biologia Molecular, Departamento de Biologia Celular, IB, University Brasilia, Brasilia, DF, Brazil.
SO Braz.J.Genet.; (1996) 19, 4, 681-82
DT Journal
LA English
AN 1997-02389 BIOTECHDS

AB A recombinant vector was constructed to display the scFv fragment of the Z22 anti-Z-DNA antibody on a filamentous phage particle surface. Phagemid pAIg 316 coding for fusion protein scFv/M13 p3 was constructed. Staphylococcus aureus protein-A promoter/leader sequences were cloned to direct the synthesis and cytolocalization of the fusion product. The cloning steps were performed by polymerase chain reaction and checked by restriction **enzyme digestion** and nucleotide sequencing. The fusion protein was detected by Western blot hybridization. The vector was tested for virus particle **production**. This, pAIg 316-transformed Escherichia coli were infected with helper phage M13k07 and the culture supernatants were analyzed by Western blot to show the fusion protein. The particles were also seen by transmission electron microscopy and the scFv fragment was detected on the particle end using Z22 Fab-specific serum and colloidal gold protein-A. This approach will permit Z22 paratope fine mapping and the **production** of novel **antibody fragment** forms with different specificities. (0 ref)

L12 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN
AN 1995:354547 CAPLUS
DN 122:130999
TI **Production** and use of **antibody fragments** to TNF-α or tricyclic antidepressant
IN Landon, John
PA Therapeutic Antibodies Inc., UK
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9429348	A2	19941222	WO 1994-GB1210	19940603
	W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	CA 2163032	AA	19941222	CA 1994-2163032	19940603
	CA 2163032	C	20010206		
	AU 9468525	A1	19950103	AU 1994-68525	19940603
	EP 703925	A1	19960403	EP 1994-917088	19940603
	EP 703925	B1	19990818		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	AT 157100	E	19970915	AT 1994-917087	19940603
	ES 2108460	T3	19971216	ES 1994-917087	19940603
	EP 916678	A3	19990526	EP 1998-203383	19940603
	EP 916678	A2	19990519		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
	AT 183513	E	19990915	AT 1994-917088	19940603
	ES 2138662	T3	20000116	ES 1994-917088	19940603
	US 5733742	A	19980331	US 1995-256053	19950310
	GR 3031415	T3	20000131	GR 1999-402504	19991007
PRAI	GB 1993-11507	A	19930603		
	GB 1994-2593	A	19940210		
	EP 1994-917088	A3	19940603		
	WO 1994-GB1210	W	19940603		

AB A method of preparing polyclonal Ig Fab fragments comprising cleaving Ig mols. with (immobilized) enzyme (e.g. papain or chymopapain), characterized in that the said cleavage is carried out on Igs in blood, serum or plasma in a closed, sterile environment. The **antibody fragments** are specific for TNF-α, tricyclic antidepressant, or nortriptyline. In example, anti-TNF-α antibody was prepared, papain and chymopapain were purified and/or immobilized for Ig digestion,

and the digested Ig fragments were purified by cation exchange chromatog.

L12 ANSWER 9 OF 9 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 1986-02952 BIOTECHDS
TI Monoclonal antibodies, electrophoretically transferred from polyacrylamide gels, retain their ability to bind specific antigens; monoclonal **antibody fragment** transfer to nitrocellulose paper for Western blotting
AU Mather S; Taylor-Papadimitriou J
LO Imperial Cancer Research Fund, P O Box 123 Lincoln's Inn Fields, London WC2A 3PX, U.K.
SO Biochem.Biophys.Res.Commun.; (1985) 133, 3, 1020-25
CODEN: BBRCA9
DT Journal
LA English
AN 1986-02952 BIOTECHDS
AB Monoclonal antibodies HC33, HC46 and ST254 react with human interferon-alpha, and were obtained from hybridomas derived from mice immunized with a mixture of human alpha-interferons purified from Sendai virus induced Namalwa human lymphoblastoid cells. All are IgG1. Monoclonal antibodies HMFG-1 and HMFG-2 were raised against human milk fat globule. Antibodies were subjected to electrophoresis on 10% isocratic polyacrylamide gel in the presence of 2% SDS, with an applied voltage of 100-150 V. Transfer to nitrocellulose was performed in a Biorad 'Trans-blot' cell using Tris-glycine buffer, pH 8.5, containing 20% methanol but no SDS. 50 V **producing** 150 mA was applied for 1-15 hr to permit transfer. The nitrocellulose was then blocked and dried, and bound radioactivity was detected by autoradiography. The antibodies retained their ability to bind specific antigen following the electrophoresis and transfer to nitrocellulose paper. Immunoreactive **antibody fragments** produced by **protease digestion** were also identified on Western blots. (21 ref)

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(FILE 'HOME' ENTERED AT 11:05:41 ON 19 JUN 2006)

FILE 'CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ESBIOBASE' ENTERED AT 11:06:24 ON 19 JUN 2006

L1 0 S (ANTIBODY FRAGMENT) AND CHO AND PEPSIN
L2 681 S (ANTIBODY FRAGMENT) AND CHO
L3 1487 S (ASPARTYL PROTEASE) OR (ASPARTYL ENZYME)
L4 2 S L2 AND L3
L5 1 DUPLICATE REMOVE L4 (1 DUPLICATE REMOVED)
L6 8803 S (ENZYME DIGESTION) OR (PROTEASE DIGESTION)
L7 1 S L2 AND L6
L8 2 S (ENDOGENOUS ENZYME) AND L2
L9 1 DUPLICATE REMOVE L8 (1 DUPLICATE REMOVED)
L10 3041 S ((ANTIBODY FRAGMENT) AND (PRODUCTION OR PRODUCING))
L11 12 S L6 AND L10
L12 9 DUPLICATE REMOVE L11 (3 DUPLICATES REMOVED)